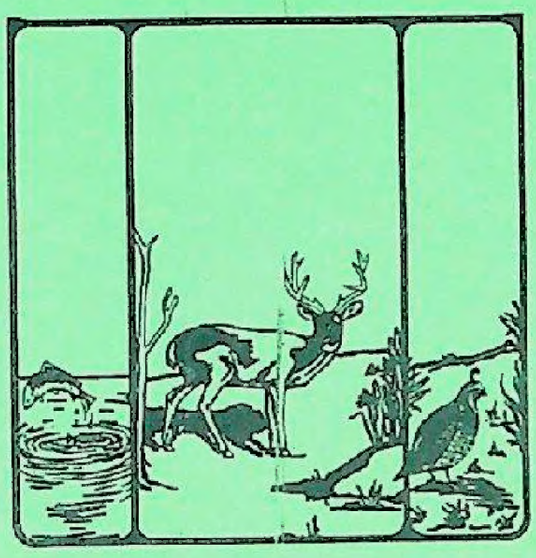
CALIFORNIA FISH and GAME



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Tui Chub, *Gila bicolor*

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DNA-BASED GENETIC MARKERS IN BLACK-TAILED AND MULE DEER FOR FORENSIC APPLICATIONS

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Forensic-quality DNA-based genetic markers have been developed for the identification of individual black-tailed deer, *Odocoileus hemionis columbianus*, and mule deer, *O. h. hemionis*, from tissue samples. The markers contain tetranucleotide short tandem repeat elements that are sufficiently polymorphic to permit identification and exclusion of identity of DNA obtained from tissue samples involved in forensic casework. Twenty-one loci were isolated and characterized from partial genomic libraries of mule deer DNA enriched for tetranucleotide motifs and cloned in *Escherichia coli*. Eight loci were selected for use as forensic markers in 4 duplex reactions that collectively provide a probability of identity of 7.7×10^{-11}, based on allele frequencies in an initial reference set of 602 animals from throughout California. Allele numbers averaged 10.25 and ranged from 5 to 11. All 4 of the duplex reactions amplify under identical conditions.

INTRODUCTION

Over the past several years, DNA-related biotechnology has grown exponentially, fundamentally driven by the potential benefits that it can provide to human health and well-being. As is the case with many rapidly developing technologies, unexpected applications have become apparent. An example of this phenomenon is the application of DNA technology to forensic science, where characterization and comparison resulting in exclusion or high probability of association of both victims and perpetrators has often been made possible by the unique features of every individual's DNA (Inman and Rudin 1997, Goldstein and Schlötterer 1999).

Most applications of forensic DNA technology have been confined to casework involving crimes by humans against humans. In contrast, the use of DNA technology in wildlife forensic situations has been minimal and the work that has been done has been modified from genetic elements developed in the context of ecological and domestic animal studies. The only case that we are aware of where forensic-grade DNA-based tools have been developed specifically for routine use in wildlife forensic applications are the procedures of Gilson et al. (1998).

Part of the reason for this deficiency has undoubtedly been the cost of creating forensic-quality markers, the paucity of funding in most governmental (federal or state) wildlife forensic laboratories, the lack of DNA-related expertise in most wildlife agencies, the need to develop separate systems for each species, and a bias toward human biomedical research. When funds are limited, more traditional uses generally receive higher priority than either molecular biology or forensic development for wildlife purposes. Consequently, the wildlife forensic community has had to rely on other, less-powerful, means of investigating crimes, including the illegal take of wildlife, or poaching.

However, DNA technology probably has greater potential use for wildlife cases than it does for casework involving humans, because 1) 2 or more animals are often taken, giving the exclusionary ability of DNA technology greater significance by determining exactly how many individuals were poached; 2) the perpetrators usually keep and preserve physical evidence for subsequent consumption or display, thus properly maintaining it for later analysis; 3) the quantities of DNA present in frozen meat and other bulk animal parts are orders of magnitude greater than is typically encountered in homicides and rapes; 4) wildlife that are contained and farmed can be, through regulation, adjusted to "genetically tag" them, enabling them to be identified from their genotypes and discriminated from wild stock restricted from market take; and 5) the number of different species involved in these crimes is limited only by human ingenuity and taste. Additionally, every forensic DNA system developed for a taxonomic group can provide immediate applications for management purposes. These include discovery of population boundaries, gene flow, level of genetic diversity, evolutionary history, and objective evidence identifying animals causing depredation or attacking humans. When either management biologists or forensic biologists develop these systems, both groups reap the benefits.

Recent developments in DNA technology and the discovery of useful genetic elements called microsatellites in eukaryotic genomes are useful in forensic science, where they can serve as powerful tools. Short tandem repeats are simple DNA sequences (e.g., TTA, TAAG, CA) that are repeated several times at many points in an organism's DNA. The number of repeat units present at a particular locus is variable. For example, a microsatellite at 1 point in an animal's DNA may have 8 repeats of the sequence TTA in 1 case and 10 repeats at the same point in the homologous chromosome. Each specific number of repeats represents an allele, and each individual animal can possess no more than 2 different alleles at a given locus. There may be many different numbers of repeat units at that locus in the population as a whole. The alleles of each locus are identified by polymerase chain reaction (PCR) amplification (Saiki et al. 1985), using primers complementary to unique sequences in the regions flanking the microsatellite. There are hundreds of thousands of different microsatellite loci in the DNA of higher organisms. The high number of loci and the high frequency of polymorphism among the loci make microsatellites useful as genetic markers for identification of individuals, demonstrating differences between individuals, and establishing geographical genetic boundaries.

The specific motifs present in a genome depend on the species. In our experience while developing microsatellite-enriched libraries in over 100 species (K. Jones),

CA enrichments are the most readily isolated, suggesting that they are generally the most common. Beckman and Weber (1992) report that CA microsatellites are about twice as common as AT microsatellites and 3 times as common as GA microsatellites in the human genome. We have observed a wide variety in the frequency of various tri- and tetranucleotide microsatellite motifs and those that are obtained most readily in 1 species may be present in low frequency or even absent in libraries prepared from other species. Microsatellites with the sequences TAGA and CATC are often readily isolated (K. Jones, personal observation). Little is known about the frequencies of microsatellites that consist of motifs with greater numbers of nucleotides because the number of different motifs rises rapidly as the number of bases in the motif increases, making it impractical to find relatively common ones. Consequently, most studies use di-, tri-, or tetranucleotide-containing microsatellites.

Tetranucleotide-containing microsatellites are useful for forensic application because the spacing between bands on an electrophoresis gel allows ready identification of specific alleles (Kimpton et al. 1994). Polymerase chain reaction amplification of tetranucleotide motifs is less prone to the production of artifactual bands differing by 1 or more repeat units (stuttering) than are di- or trinucleotide-containing microsatellites. The relative clarity of the results obtained using tetranucleotides is a significant advantage. Tetranucleotide-based microsatellites are currently available commercially for use in human forensics and paternity testing (Micka et al. 1999), but, to our knowledge, none are available commercially for wildlife.

In California, the number of black-tailed deer, *Odocoileus hemionis columbianus*, and mule deer, *O. h. hemionis*, harvested legally each year is about 30,000. The number of deer killed by poachers is thought to be about twice that, although precise numbers are obviously difficult to determine (Wildlife Protection Division, California Department of Fish and Game, personal communication). The ability to show identity, lack of identity, and population source among forensic samples of black-tailed and mule deer would be of value in wildlife law enforcement. We report here on the development of 21 tetranucleotide-containing microsatellites and characterization of 8 that appear to be suitable for routine forensic use. We also report on the use of a relatively low-cost electrophoretic system that provides the resolution necessary to identify the alleles of each of these microsatellite-containing loci. A standard operating procedure for the use of these loci is available from the junior authors.

METHODS

Library Preparation

DNA was extracted and purified from deer muscle tissue using commercial spin columns (QIAGEN Inc.[1], Valencia, California, USA). DNA was eluted from the columns with 125 µliter Tris buffer, pH 9.0, and stored at –20°C. Concentration and purity was determined by UV spectroscopy and agarose gel electrophoresis. Agarose

[1] Reference to trade names does not imply endorsement by the California Department of Fish and Game.

electrophoresis (1%, TAE) showed the DNA to be primarily high molecular weight with a modest amount of degradation and slight RNA contamination.

DNA was partially digested with a mixture of 8 blunt end-cutting restriction endonucleases. Digestion products were separated by agarose electrophoresis for a period sufficient to produce a smear of fragments when visualized by ethidium bromide staining. Fragments in the range of 325–650 bp were recovered from the gel (QIAEX II, QIAGEN Inc.[1]), adapted with 20-nucleotide oligonucleotides that contained a PCR anti-primer sequence and a *Hin*d III restriction site. The mixture of fragments was subjected to magnetic bead capture using biotinylated oligonucleotides consisting of various tetranucleotide motifs (CPG, Inc.[1], Lincoln Park, New Jersey, USA). Captured molecules were amplified by PCR using sequences complementary to the adapters as primers. Fragments were restricted with *Hin*d III and ligated into the *Hin*d III site of plasmid pUC19 (Sambrook et al. 1989). Ligated plasmids were then electroporated (BTX Transporator, 12.25 kV/cm, 1-cm path) into 40 µliter *E. coli* DH5α. Transformed cells were diluted to 1 ml and allowed to recover for 1 hour at 37°C. One-tenth ml aliquots were plated onto LB agar plates supplemented with Bluogal, IPTG, and ampicillin (Gibco BRL Products[1], Grand Island, New York, USA).

DNA Sequencing

Individual white (recombinant) colonies were selected arbitrarily from culture plates on which 0.1 ml aliquots of a library had been plated. A portion of each colony was transferred to individual squares of a grid placed behind a fresh LB agar plate supplemented with Bluogal, IPTG, and ampicillin. After overnight growth, portions of the sub-cloned colonies were transferred to a PCR tube containing PCR master mix. The tubes were tightly sealed and heated to 100°C for 3 minutes. After BIOTAQ DNA Polymerase (Bioline USA Inc.[1], Reno, Nevada, USA) was added, amplification was carried out using pUC19 universal primers. Primer sequences were 5'- AGG AAA CAG CTA TGA CCA TG -3' (forward) and 5'- ACG ACG TTG TAA AAC GAC GG -3' (reverse). Amplification products were then separated and sized by agarose gel electrophoresis. Clones that produced fragments in the size range of 300–700 bp were selected for sequencing, avoiding selection of clones that produced fragments of similar length. Plasmids were recovered from the clones and sequenced using Prism Cycle Sequencing Kits and labeled dNTPs (PE-Applied Biosystems, Inc.[1], Foster City, California, USA). Sequences were obtained on a PE-Applied Biosystems Model 373A DNA sequencer.

PCR Primer Design and Testing

A few dozen microsatellite-containing loci were identified that appeared to have flanking sequences suitable for PCR primer design, based on visual inspection of the sequencing results. The criteria for selection included the proximity of the microsatellite to the insertion site in the vector, the degree of degradation of the microsatellite locus itself, and the proximity of other repeat motifs (e.g., single-base runs, dinucleotide microsatellites) to the intended locus. Commercial software

(Designer PCR, Research Genetics, Inc.[1], Huntsville, Alabama, USA) was then used to design primer pairs. Primers that satisfied stringency requirements were purchased from a commercial DNA synthesis company (Integrated DNA Technology, Inc.[1], Coralville, Iowa, USA). Primer pairs were diluted to appropriate concentration and tested for their ability to support amplification from the original clone and from 6 to 8 samples of genomic DNA. Polymerase chain reaction conditions are described in Table 1.

Identification and Characterization of Polymorphic Loci

PCR amplification products were initially separated on 4% agarose gels and visualized by ethidium bromide-based fluorescence to determine whether or not multiple products were produced. Polyacrylamide electrophoresis was carried out on 0.4-mm thick, 6% polyacrylamide, 24-cm well-to-read gels in a PE-Applied

Table 1. Polymerase chain reaction conditions for duplex reactions of genomic DNA for California black-tailed and mule deer.

Reaction mix:

Component	Stock concentration	Final concentration	Volume/reaction
H_2O			4.55 µl
Biolase buffer	10X	1X	1.0 µl
Sucrose/cresol red	10X	1X	1.0 µl
$MgCl_2$	50 mM	2 mM	0.4 µl
dNTP's (premixed)	2.5 mM each	0.2 mM each	0.8 µl
Primers (premixed)	10 µM	varies, see below	1.2 µl
Biolase	5 U/µl	0.025 U/µl	0.05 µl
Template DNA	10 ng/µl	1 ng/µl	1.0 µl
Total			10.0 µl

Final primer concentration for each duplex:

Duplex	Primer pair	Concentration
C	M	0.6 µM
	P	0.6 µM
D	D	0.6 µM
	Q	0.6 µM
F	K	0.6 µM
	N	0.6 µM
G	O	0.85 µM
	R	0.35 µM

Polymerase chain reaction amplification conditions:

Initial denaturation:	94°C	3 minutes
Amplification conditions (30 cycles):		
Denaturation:	94°C	40 seconds
Annealing:	61°C	40 seconds
Extension:	72°C	30 seconds
Final extension:	72°C	4 minutes
Soak:	<28°C	

Biosystems[1] Model 373A automated sequencer. Separation was carried out for 8 hours at 40 mA and 30 W. Product lengths were determined by including ROX 500 (PE-Applied Biosystems, Inc.[1]) with each sample to serve as size standards. Products were analyzed using GeneScan Version 1.2.2 (PE-Applied Biosystems, Inc.[1]). Polymerase chain reaction products were labeled during synthesis with fluorescent dUTPs (PE-Applied Biosystems, Inc.[1]). Genotypes were recorded in FileMaker Pro Version 2.1 (Claris Corp.[1], Santa Clara, California, USA).

In addition to acrylamide electrophoresis on denaturing gels, we identified PCR products using preformed gel matrices in a submerged horizontal native (non-denaturing) format in a temperature-controlled electrophoresis unit (Spreadex EL 600, Elchrom Scientific (USA), Inc.[1], Lake Park, Florida, USA). Four-base-pair resolution is readily achieved in all portions of the gel where alleles may be expected to occur following electrophoresis. Electrophoresis was conducted at 120 v for 2.5–3.5 hours. To visualize PCR products, gels were soaked in a 0.01% (vol/vol) solution of SYBR Gold (Molecular Probes, Inc.[1], Eugene, Oregon, USA), a DNA-specific fluorescent dye, and viewed on a standard UV transilluminator at 312 nM. Gels were photographed and the position of the bands compared with standards or from sample to sample to identify alleles.

RESULTS

Approximately 100 µg of high molecular weight DNA was extracted from muscle tissue of a single deer. The extracted and purified DNA was of relatively high molecular weight, as determined by agarose electrophoresis; its A_{260}/A_{280} absorbance ratio was about 1.85, indicating that it was relatively free of protein and other contaminants that absorb in this range of the ultraviolet spectrum. Suitability of the quality of the DNA for further work was confirmed by demonstrating that the DNA could be cleaved with restriction endonucleases.

The DNA was of suitable quality to cut with a mixture of restriction enzymes which, when taken to completion, reduced the fragments to less than about 350 bp. Partial digestion gave a range of fragment lengths that appeared as a smear through the entire length of an agarose gel.

Recombinant plasmid containing cells were identified by standard blue-white selection and white colonies were subcloned (Sambrook et al. 1989). Two enriched libraries were successfully prepared, using the motif $(CATC)_n$ in 1 case, and a mixture of $(TAGA)_n$, $(TGGA)_n$, and $(TAAA)_n$ as capture molecules in the 2nd case. Each of the transporation products yielded 1.4 ml of transformed *E. coli*, containing about 10^3 cells/ml. The 1st library was enriched to about 80%, and the 2nd to about 94%, with the majority of the microsatellites consisting of $(TAGA)_n$.

Twelve clones were arbitrarily selected from each preparation for sequencing. On the basis of these results, 171 clones from the 2 libraries were selected for additional sequencing.

Forty-two microsatellite-containing clones were selected for PCR primer design on the basis of location of the microsatellite motif relative to insertion point in the plasmid, complexity, and integrity of the motif. Typical design parameters are shown

in Table 2. Twenty-nine loci for which primer pairs were suggested by the software were selected and synthesized (Integrated DNA Technology, Inc.[1]).

Primer pairs that supported amplification from clonal DNA were tested for their ability to support amplification from genomic DNA, again using the standard amplification conditions of Table 1. Reactions were carried out generally using DNA from 4–6 individuals and the products separated by electrophoresis on 3.5% agarose gels. Twenty-seven (93%) of the primer pairs supported amplification of product of appropriate length as determined from the original clonal sequence. Resolution was sufficient in this system to identify loci that were grossly polymorphic.

Initially, DNA was extracted from tissues of 24 deer and used as a template for microsatellite amplification using 21 PCR primer pairs. The deer came from throughout California in order to increase the likelihood of detecting polymorphism. These sequences have been submitted to GenBank (www.ncbi.nlm.nih.gov/Genbank) and have been assigned accession numbers AF102240–AF102260.

Allele frequency distribution for each locus was determined using FileMaker Pro scripts (Paetkau et al. 1995). Twenty-one polymorphic loci were characterized (Fig. 1). Based on the distribution of allele sizes for each locus and the probability of identity calculated for each locus, pairwise combinations of loci were selected for co-amplification and electrophoresis. Loci were combined that showed a maximum gap between the smallest allele of the larger locus and the largest allele of the smaller locus in every pairwise combination. Maximum spacing was desired to allow for additional alleles that might be discovered as more animals are genotyped.

Loci initially tested in pairs sometimes failed to amplify satisfactorily, so several combinations were tried until combinations that could be amplified under a single set of conditions were found. Primers were redesigned in some cases to provide a larger space between loci. Ultimately, 4 pairs of loci were identified that could be

Table 2. Typical polymerase chain reaction primer design parameters for California black-tailed and mule deer.

Primer length minimum (bases)	18
Primer length maximum (bases)	22
Primer Tm minimum (°C)	55
Primer Tm maximum (°C)	60
Primer Tm Variance (°C)	+1
Primer %GC minimum	35
Primer %GC maximum	65
Primer concentration (µMol)	50
Salt concentration (mMol)	50
Product length minimum (bp)	100
Product length maximum (bp)	300
Eliminate:	
Hairpins with stem ≥	4
Dimers with Tm ≥	22
3'/template homology ≥	5
3' dimers ≥	2

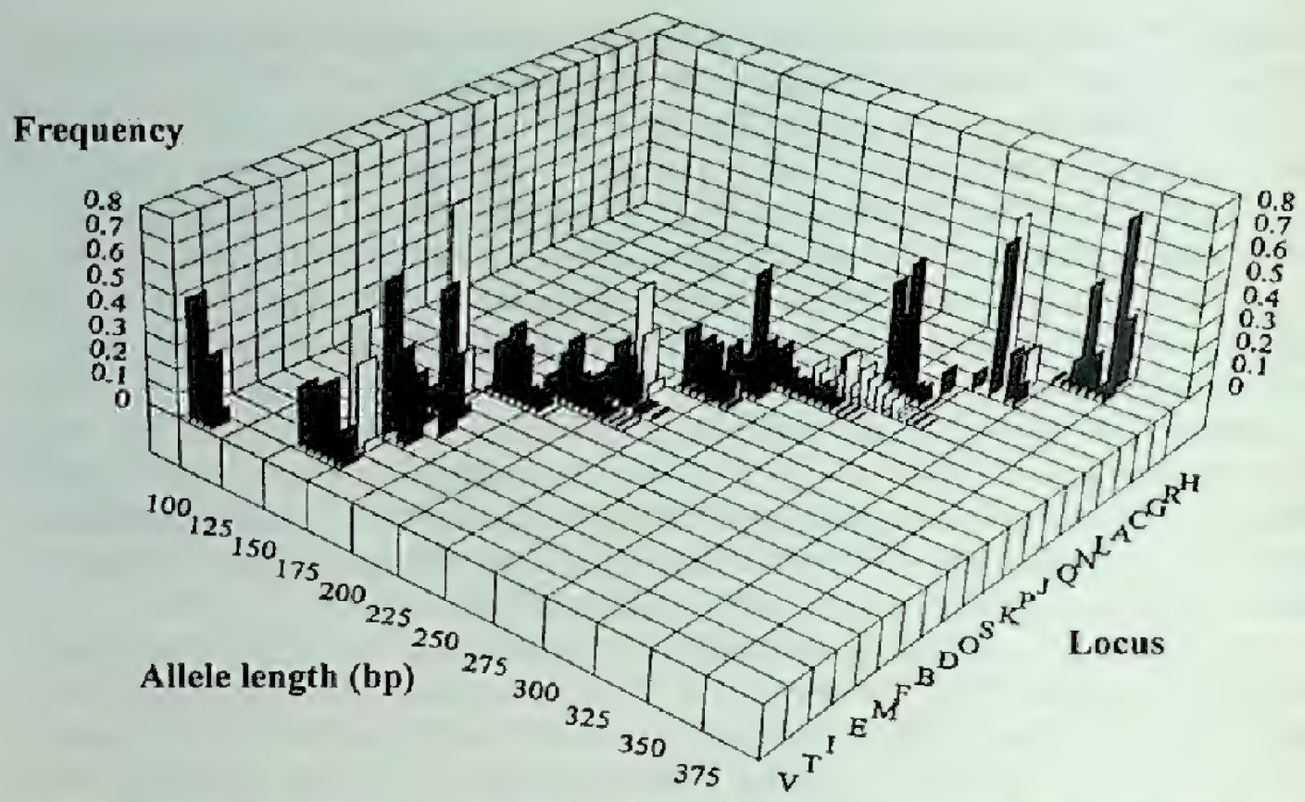

Figure 1. Twenty-one polymorphic tetranucleotide loci for black-tailed and mule deer from California. Loci are sorted by minimum allele length. (N = 602 for the California Department of Fish and Game suite of 8 loci and N = 24 for the remaining 13 loci). Each cluster of bars represents a specific locus and each bar represents an allele of the locus. The position of a bar indicates the length of the PCR product from the allele and the height of a bar indicates the frequency of the allele.

amplified under a single set of conditions and were separated upon gel electrophoresis by distances that should allow unambiguous identification of new alleles that may be discovered in the future (Table 3).

After the 8 loci were selected, the reference set was expanded to 602 animals. The allele frequencies and size distributions of the 4 pairs of loci in the set of 602 animals are listed in Table 4 and illustrated in Fig. 2. There was an average of 10.25 alleles per locus (range 5–15). The probability of identity between 2 animals picked at random with respect to these 8 loci is approximately 7.7×10^{-11}, or 1 in 13 billion (Paetkau et al. 1995).

We have tested these loci using 4 different electrophoresis methods and find them to give unambiguous results in every case. These include 4% agarose electrophoresis, native (non-denaturing) polyacrylamide electrophoresis, denaturing polyacrylamide gel electrophoresis with fluorescent dUTP labeling, and denaturing polyacrylamide gel electrophoresis with dye-labeled PCR primers.

DISCUSSION

In this report, we have described a set of molecular markers that promises to be suitable for providing evidence for the identity (or non-identity) of tissues obtained from black-tailed and mule deer. The markers are of a class termed microsatellites.

Table 3. Primer pairs used for PCR amplification of California black-tailed and mule deer samples. Designations preceding primer sequences are the clone sequences from which the primers were designed.

Duplex C:	
Locus M	
DeerC 273-F	5'-AGG GAA ACC TCT GTT CAG GA-3'
DeerC 273-R	5'-ACC AAG CAA AAT GCC TTA CA-3'
Locus P	
DeerT 7-F	5'-TTT CAC TGT TTT CTC CTT CAG A-3'
DeerT 7-R	5'-TGC CCA ATC AGA TGT TGT AG-3'
Duplex D:	
Locus D	
DeerC 89-F	5'-AGA GCC TCG TCT TTT CAT TC-3'
DeerC 89-R	5'-TTG CTG CTT GCT TGT CTA AT-3'
Locus Q	
DeerT 32-F	5'-AAT GTG TCA GTG AAG GTC TTC-3'
DeerT 32-R	5'-ATC CAG GCA ACC ATC TAG-3'
Duplex F:	
Locus K	
DeerT 217-F	5'-GCA GGA AGG AGG AGA CAG TA-3'
DeerT 217-R	5'-GCT GGT TCG TTA TCA TTT AGC-3'
Locus N	
DeerT 27L-F	5'-GCA ACC AAT AGG ATA GGT CG-3'
DeerT 27L-R	5'-GCT GGA TGG AAC TGA AAG TC-3'
Duplex G:	
Locus O	
DeerT 159S-F	5'-ACG AGG TTC AGT GGT TCC-3'
DeerT 159S-R	5'-CAG GGC ATA GTT TCC AAA-3'
Locus R	
DeerT 106A-F	5'-GGG GTC TTC TCA ATC CA-3'
DeerT 106A-R	5'-TCA GTT TCT GGA ACT CTA AAG T-3'

Based on the genotypes of a reference sample of 602 mule deer from throughout California, the 8 microsatellite loci described here appear to provide a level of resolution more than adequate to identify every individual deer in California (with the exception of monozygotic twins). The loci can be amplified using a single set of PCR conditions and individual alleles are readily identified by gel electrophoresis, so long as the electrophoresis matrix has the capability of resolving 4-base differences in the size of PCR amplification products.

These markers can be used as forensic tools. Their use in an exclusionary context provides proof of the existence of multiple individuals in evidentiary samples and also demonstrates the non-identity of samples when such is the case. As with similar markers used in human forensics, certain other questions remain to be answered concerning these markers. Chief among these is whether the markers provide sufficient resolution among deer in relatively small, and potentially inbred, populations. The probability of identity between any 2 deer taken at random can be

Table 4. Allele frequencies for each of the 8 microsatellite loci in a sample of 602 California black-tailed and mule deer.

Duplex C				Duplex D				Duplex F				Duplex G			
Locus M		Locus P		Locus D		Locus Q		Locus K		Locus N		Locus O		Locus R	
Allele	Freq.	Allele	Freq.	Allele	Freq.	Allele	Freq.	Allele	Freq.	Allele	Freq.	Allele	Freq.	Allele	Freq.
03	0.160	02	0.004	02	0.023	02	0.010	03	0.145	03	0.076	02	0.077	03	0.081
04	0.284	03	0.265	03	0.218	03	0.107	04	0.126	04	0.063	03	0.076	04	0.000
05	0.000	04	0.017	04	0.042	04	0.169	05	0.428	05	0.031	04	0.197	05	0.000
06	0.197	05	0.236	05	0.311	05	0.100	06	0.246	06	0.068	05	0.265	06	0.000
07	0.029	06	0.220	06	0.220	06	0.184	07	0.056	07	0.173	06	0.119	07	0.012
08	0.000	6.5	0.002	07	0.058	07	0.082			08	0.061	07	0.087	08	0.044
09	0.000	07	0.122	08	0.061	08	0.051			09	0.141	08	0.087	09	0.178
10	0.331	08	0.114	09	0.066	09	0.062			10	0.108	09	0.042	10	0.347
		09	0.018	10	0.001	10	0.041			11	0.092	10	0.025	11	0.206
						11	0.071			12	0.075	11	0.011	12	0.059
						12	0.074			13	0.062	12	0.012	13	0.066
						13	0.030			14	0.022	13	0.002	14	0.007
						14	0.009			15	0.015				
						15	0.007			16	0.008				
						16	0.002			17	0.003				
No. alleles	5		9		9		15		5		15		12		9
Prob. identity	0.109		0.072		0.071		0.022		0.120		0.018		0.039		0.072

Overall probability of identity is 7.7×10^{-11}, or 1 in 1.3×10^{10}. Mean number of alleles is 10.25.

The probability of identity for each locus is $I = \sum_i p_i^4 = \sum\sum_{i\ j} (2p_i p_j)^2$, where p equals the probability of alleles i through j for a given locus. Overall probability of identity is the product of the probabilities of identity for all loci.

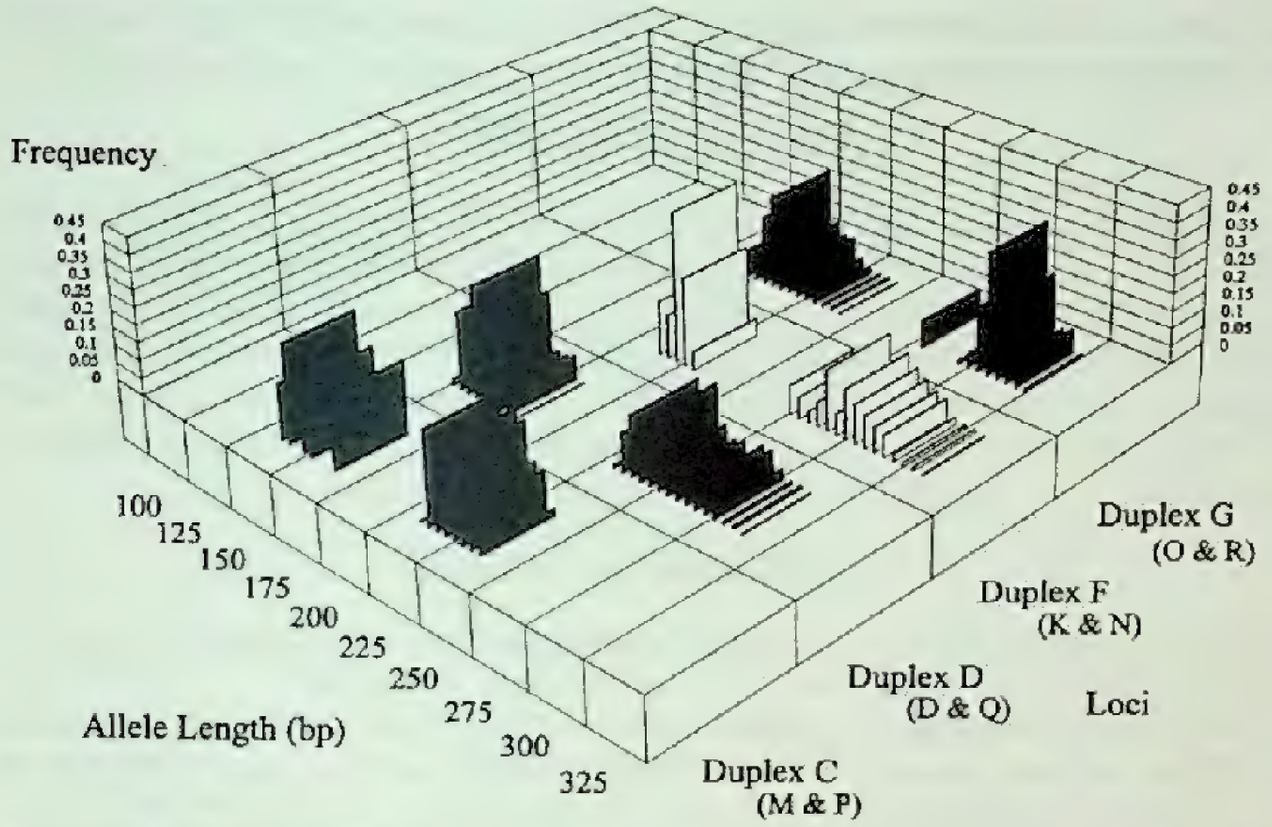

Figure 2. Allele sizes and frequencies in California Department of Fish and Game suite of 8 polymorphic tetranucleotide loci in black-tailed and mule deer from California (N = 602). Eight of the 21 loci shown in Fig. 1 were combined in 4 duplex reactions. Loci in a duplex are PCR amplified in the same reaction and separated in a single electrophoresis lane.

readily calculated using well-established formulas. However, these values are based on frequencies of the various alleles identified in the reference set. Whether or not similar frequencies exist among animals from more localized regions remains to be determined and, based on results obtained from human and wildlife populations, each population will probably be found to have its own unique and characteristic set of allele frequencies.

Perhaps a more useful perspective for the judicial process is whether or not any 2 animals taken from a region can be shown to have the same genotype with respect to these loci. That is, rather than expressing the high level of resolution provided by these markers with the results of an arcane probability calculation based on assumptions that may or may not be proven, it may be more useful to demonstrate simply that no 2 identical genotypes have been found among California deer. Obviously, this will require the genotyping of a suitably large set of animals, containing numerous individuals from throughout the state. To this end, we have begun genotyping several hundred additional deer from precisely known geographical locations. Initial results have demonstrated significant differences in allele frequency in animals from various regions.

Genotyping more animals will have other benefits. For example, data obtained from such work will probably provide insights into the population structure of black-tailed and mule deer as a whole. That information may be useful in

understanding the population dynamics of the species and in developing regulations and management policies.

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SURVIVORSHIP AND CAUSE-SPECIFIC MORTALITY IN SYMPATRIC POPULATIONS OF MOUNTAIN SHEEP AND MULE DEER

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We examined survivorship and cause-specific mortality in sympatric populations of mountain sheep, *Ovis canadensis*, and mule deer, *Odocoileus hemionus*, inhabiting a remote region of California. Predation by mountain lions, *Puma concolor*, was confirmed in 75% (n = 9) of sheep deaths and 55% (n = 5) of investigated deer deaths. Overall, sheep and deer survived at nearly identical rates; however, female sheep survived at a significantly higher rate than did males, consistent with the hypothesis that differential use of habitats by gender results in greater risk to males. In California, there are indications that impacts by mountain lions to mountain sheep populations are restricted to areas where mountain sheep and mule deer are sympatric.

INTRODUCTION

Understanding the causes of mortality and survival rates for hunted and non-hunted populations of wild ungulates is important for their proper management and conservation. Although legal harvest commonly is estimated by resource management agencies, non-hunting mortality is less-often measured. Knowledge of specific causes of mortality may be especially important for managing populations of mountain sheep, *Ovis canadensis*, which have a naturally fragmented distribution (Bleich et al. 1990) and can occur sympatrically with other native ungulates such as mule deer, *Odocoileus hemionus* (Hornocker 1970). These often small and disjunct populations are vulnerable to a variety of mortality factors that individually, or in combination, can lead to uncertain probabilities of persistence (Berger 1990).

In California, mountain sheep have declined or been extirpated over much of their range (Torres et al. 1994). Populations of mountain sheep occurring even in

proximity to each other can exhibit markedly different dynamics (Stevens and Goodson 1993), a potentially important consideration when implementing conservation strategies.

Declining populations of mule deer have been of long-term concern throughout the western United States (Longhurst et al. 1976, Workman and Low 1976). Factors potentially contributing to these declines are decreasing trends in the availability of early seral vegetation (Gruell[1] 1986), loss and fragmentation of habitats (Reed 1981), changing climatic conditions (Longhurst et al. 1976), diseases (Woods et al. 1996), and predation (Bleich and Taylor 1998). The relative importance or temporal relationships of those factors in regulating deer numbers is not well understood.

In some areas where the distributions of mountain sheep and mule deer overlap, predation, especially by mountain lions, *Puma concolor*, has been reported to be an important source of sheep mortality (Jaeger[2] 1994, Wehausen 1996), but specific causes and rates of mortality among most populations of mountain sheep are poorly known. An understanding of cause-specific mortality and survivorship in mountain sheep populations may be useful when developing management objectives for the protection or re-establishment of wild sheep on native ranges. Also, knowledge of mortality and survivorship in specific populations of mule deer could provide a better understanding of factors influencing productivity and would be useful in assessing current trends and impacts to populations.

Wildlife harvest strategies often rely on estimates of natural mortality. Knowledge of mortality factors facilitates understanding of population dynamics and can be useful for developing management or conservation strategies. We describe cause-specific mortality and estimate survivorship in sympatric populations of mountain sheep and mule deer in a remote region of California. Our study area provided a unique opportunity to examine differences in survival and mortality in relation to sexual segregation and use of seasonal ranges by these large herbivores.

STUDY AREA

We conducted this investigation in the Mission Creek and Whitewater River drainages of the San Gorgonio Wilderness in the San Bernardino Mountains, Riverside and San Bernardino counties, California (34°05'30"N, 116°47'00"W). The San Bernardino Mountains are one of several transverse ranges in southern California that consist of high peaks and ridges with incised, steep-walled canyons (Hickman 1993). The cismontane slopes of the study area are characterized by coastal chaparral at low elevations, with mixed oak woodlands and montane forests of white fir, *Abies concolor*; Jeffrey pine, *Pinus jeffreyi*; and lodgepole pine, *Pinus contorta*, at upper

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[1] Gruell, G.E. 1986. Post-1900 mule deer irruptions in the intermountain west: Principal cause and influences. General Technical Report INT-206, USDA Forest Service, Inter-mountain Research Station, Ogden, Utah, USA.

[2] Jaeger, J.R. 1994. Demography and movements of mountain sheep (*Ovis canadensis nelsoni*) in the Kingston and Clark Mountain Ranges, California. M.S. Thesis, University of Nevada, Las Vegas, Nevada, USA.

elevations. Low-elevation transmontane slopes support vegetation representative of the Creosote Bush Series and Joshua Tree Series (Paysen et al.[3] 1980).

Elevations in the study area range from 915 m on the eastern end of the area to >3,500 m at the summit of Mt. San Gorgonio. Climate is typified by cool winters, averaging < −10°C at upper elevations, and hot, dry summers. Annual precipitation generally exceeds 50 cm at mid-elevations, with the first snow typically occurring in November and persisting at upper elevations until June. A detailed description of the vegetation and geological diversity of the study area is provided by Light et al.[4] (1966).

METHODS

We captured adult mountain sheep and mule deer using a hand-held net gun fired from a helicopter (Krausman et al. 1985) or from the ground (R.J. Schaefer, unpublished data). We attached radio collars equipped with mortality sensors (Telonics, Inc.[5], Mesa, Arizona, USA or Lotek, Inc.[5], Newmarket, Ontario, Canada) and a colored, 6 x 8-cm plastic livestock tag (Temple Tags, Inc.[5], Temple, Texas, USA) to an ear of each animal. The status (alive or dead) of every marked animal was determined each week by ground or aerial telemetry. Upon detection, carcasses of dead animals were inspected as quickly as feasible to determine cause of death. When predation was the source of mortality, we followed Wade and Bowns (1985) or Kunkel and Mech (1994) to confirm the species of predator. When the cause of death could not be confirmed, it was categorized as undetermined; those deaths that could not be investigated in a timely manner were considered unexamined.

We used a modification of the Kaplan-Meier (1958) product-limit procedure, which allows for staggered entry of individual animals, to determine survivorship of deer and sheep (Pollock et al. 1989). We compared survival between species, and between genders within species, using the log-rank test of Cox and Oakes (1984), as modified by Pollock et al. (1989). By categorizing months as winter-spring (January–June) or summer-fall (July–December), we compared seasonal differences in natural mortality using chi-square (Zar 1984). Median survival was defined as the number of months at which 50% of the collared cohort of either deer or sheep remained alive.

RESULTS

We collared 26 mountain sheep (10 males, 16 females) and 34 mule deer (9 males, 25 females) between January 1993 and August 1995. During January 1993–May 1996 (40 months), 12 collared sheep (46%) and 17 collared deer (50%) died (Table 1).

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[3] Paysen, T.E., J.A. Derby, H. Black Jr., V.C. Bleich, and J.W. Mincks. 1980. A vegetation classification system applied to southern California. General Technical Report PSW-45, USDA Forest Service, Pacific Southwest Forest and Range Experiment Station, Berkeley, California, USA.

[4] Light, J.T., T.R. Zrelak, and H. Grahm. 1966. San Gorgonio bighorn management plan. USDA Forest Service, San Bernardino National Forest, San Bernadino, California, USA.

[5] The use of trade names does not imply endorsement by the California Department of Fish and Game.

Seventy-five percent (9 of 12) of mountain sheep deaths were caused by mountain lion predation; the cause of 3 sheep deaths could not be determined. Overall, mortality was 70% (7 of 10) for male sheep, and 31% (5 of 16) for females. Annual survivorship for mountain sheep was 0.80 and median survival was 24 months (Table 2). Median survival for male sheep (19 months) differed greatly from that of female sheep (> 40 months), and survivorship curves for male and female mountain sheep differed significantly (log-rank test statistic = 4.18, df = 1, $P < 0.05$), indicating a greater rate of mortality for males (Fig. 1). A greater proportion of mountain sheep died during winter-spring (75%) when compared to summer-fall (25%), but the difference was not significant ($P > 0.10$), possibly due to small sample sizes.

Among mule deer, mountain lions were responsible for 55% (5 of 9) of all deaths that could be investigated. Additionally, 1 deer was killed by domestic dogs, 2 deer were killed legally by hunters, and the cause of 1 deer death was undetermined. Eight mortalities were unexamined due to severe weather or because of restrictions on the use of helicopters in the wilderness area. Overall, natural mortality was 71% (5 of 7) for male deer and 38% (9 of 24) for females. Annual survivorship for deer was 0.81, and median survival was 30 months. Annual survivorship for female deer was 0.83, and >50% of females remained alive for the duration of our study (40 months). An inadequate sample size for males precluded an analysis of survivorship between sexes of mule deer.

Table 1. Causes of mortality for telemetered mule deer and mountain sheep in the Whitewater and Mission Creek drainages of the San Gorgonio Wilderness in the San Bernardino Mountains, Riverside and San Bernardino counties, California, January 1993–May 1996.

	Lions	Undetermined	Hunting	Dogs	Unexamined
Male deer (n = 9)	2	0	2	0	3
Female deer (n = 25)	3	1	0	1	5
Male sheep (n = 10)	5	2	0	0	0
Female sheep (n = 16)	4	1	0	0	0
Total	14	4	2	1	8

Table 2. Survivorship of mountain sheep and mule deer in the Whitewater and Mission Creek drainages of the San Gorgonio Wilderness in the San Bernardino Mountains, Riverside and San Bernardino counties, California, January 1993–May 1996.

	n	Monthly survival	Annual survival	Median survival (months)
Sheep	26	0.983	0.800	24
Male sheep	10	0.980	0.763	19
Female sheep	16	0.988	0.863	>40
Deer	34	0.984	0.813	30
Female deer	25	0.986	0.833	>40

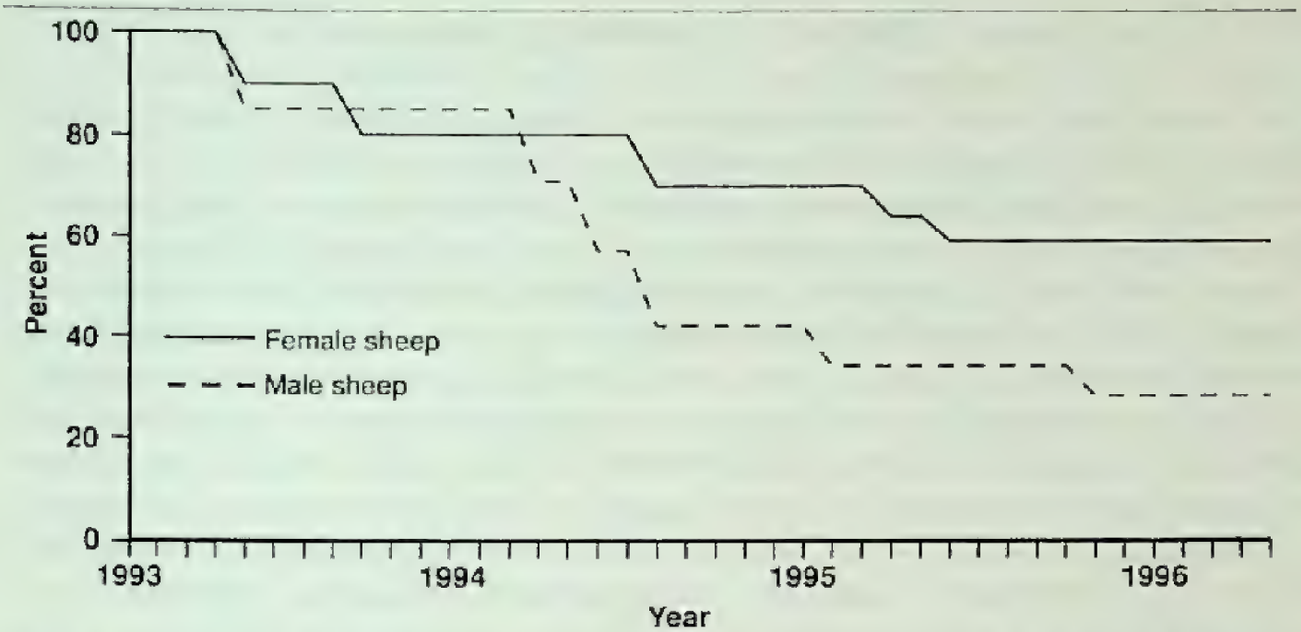

Figure 1. Survivorship of female (n = 16) and male (n = 10) mountain sheep in the Whitewater and Mission Creek drainages of the San Gorgonio Wilderness of the San Bernardino Mountains, Riverside and San Bernardino counties, California, January 1993–May 1996.

DISCUSSION

In California, available evidence suggests that mountain lions permanently inhabit areas occupied by mountain sheep only where deer occur sympatrically and at densities adequate to provide a primary source of prey for lions. For example, the Granite Mountains of California support sympatric populations of mule deer and mountain sheep, and the status of that small population of mountain sheep is closely related to the level of predation by mountain lions (Wehausen 1996). Similarly, in the Sierra Nevada, where mountain sheep are sometimes preyed upon by mountain lions (Bleich et al. 1991, Wehausen 1996), sheep are sympatric with mule deer on a number of winter ranges (Jones 1950, McCullough and Schneegas 1966, Bleich and Taylor 1998). Moreover, mule deer and mountain sheep are sympatric in other parts of California where predation by mountain lions on mountain sheep has been reported (Jaeger[5] 1994, Boyce et al.[6] 1996) or may be a factor in sheep population declines (S.A. Holl, S.G. Torres, and V.C. Bleich, unpublished data). Densities of mule deer on those ranges are not precisely known, but are thought to be adequate to support permanent mountain lion populations (Torres et al. 1996).

In contrast, Andrew et al. (1997) found no evidence of predation by mountain lions on mountain sheep in the Sonoran Desert, possibly because of the low density of mule deer (Thompson and Bleich 1993), or, alternatively, the low density of mountain lions (McIvor et al. 1995). Similarly, Bleich et al. (1997) and Oehler[7]

[6] Boyce, W., E. Rubin, C. Hayes, S. Torres, and M. Jorgensen. 1996. [Abstract] Mountain lion predation on bighorn sheep in the peninsular ranges of California. Proceedings of the Biennial Symposium of the Northern Wild Sheep and Goat Council 10:12.

[7] Oehler, M.W. Sr. 1999. Ecology of mountain sheep: Effects of mining and precipitation. M.S. Thesis, University of Alaska Fairbanks, Fairbanks, Alaska, USA.

(1999) reported low rates of predation by mountain lions on mountain sheep in parts of the Mojave Desert supporting low densities of mule deer (Longhurst et al. 1952, Welles and Welles 1961). Mountain lions may not persist where densities of deer are lower than 1.0 animal/km^2 (Shaw et al.[8] 1988, Peirce and Cashman[9] 1993). In our study, mountain sheep shared range spatially and temporally with mule deer and predation by mountain lions was the most important source of mortality for both species.

Previous studies of populations of mountain sheep and mule deer have shown that predation by mountain lions is a factor influencing rates of survival and mortality. Our study found rates of mortality and survival similar to those in other populations of mountain sheep and mule deer that were preyed upon by mountain lions in California. In the Granite Mountains, 55% (5 of 9) of collared female mountain sheep were killed by mountain lions; annual survivorship ranged from 0.625 during years mountain lions killed collared sheep to 1.00 during years they did not (Wehausen 1996). Among 9 collared sheep in the Kingston and Clark ranges, 67% died as a result of predation by mountain lions (Jaeger[2] 1994). In a retrospective analysis of 5 populations of mule deer (N = 168 collared animals) in the western Great Basin, where predation was the leading cause of death, annual survival ranged from 0.643 to 0.884 and mountain lions were responsible for 90% of deer killed by predators (Bleich and Taylor 1998).

We found that male mountain sheep survived at a significantly lower rate than females and, overall, mortality for deer and sheep was greater for males than females. These patterns of increased mortality among males is consistent with the hypothesis that females increase their chances for survival by foraging in less risky habitats than males (Bowyer 1984, Main and Coblentz 1996, Bleich et al. 1997).

We cannot discount the possibility that predation rates we estimated were biased by the presence of conspicuous plastic ear tags. Anything that makes an individual appear "different" to a predator may influence rates of predation (Curio 1976). In our study, ear-tagged animals were often detected by sunlight reflecting from the plastic ear tags or from ear flicks by marked individuals (R.J. Schaefer, personal observation). We assumed that the effect of ear tags on predation rates was similar for deer and sheep; however, differences in diel activity patterns between these species could result in differential vulnerability to predation. It may be important to consider the potential effects of conspicuous markings, especially ear tags, used to study wild ungulates when extrapolating predation rates, and their resultant effects on survivorship, to the level of the population.

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[8] Shaw, H.G., N.G. Woolsey, J.R. Wegge, and R.L. Day, Jr. 1988. Factors affecting mountain lion densities and cattle depredation in Arizona. Final Report, Federal Aid in Wildlife Restoration Project W-78-R, Arizona Game and Fish Department, Phoenix, Arizona, USA.

[9] Peirce, M. and J. Cashman. 1993. Region IV mountain lion project. Final Report, Federal Aid in Wildlife Restoration Project W-53-M-43, Arizona Game and Fish Department, Phoenix, Arizona, USA.

MANAGEMENT IMPLICATIONS

In the San Bernardino Mountains, urbanization has reduced the amount of high-quality winter habitat (Nicholson[10] 1995) and resulted in high densities and substantial spatial overlap of mountain sheep and mule deer. This concentration of prey on remaining winter range may be an important factor contributing to high risks of predation for both species. Of all sheep mortalities, 75% (N = 9) occurred during winter–spring, when seasonal densities of both species was highest. Wehausen (1996) suggested that increasing mountain lion activity may have caused the abandonment of low elevation winter ranges by bighorn sheep throughout the southern and central Sierra Nevada Mountains. Pierce et al. (in press) recently demonstrated that the distribution and availability of prey, and not intraspecific social mechanisms, likely regulate mountain lion populations in specific geographic areas. Loss and contraction of winter ranges result in a more predictable and temporarily higher-density prey base, where the efficiency of predation by mountain lions may be enhanced.

Alternate prey, such as mountain sheep, that are at risk of significant impacts from predation may require special management if the objective is to maintain viability of protected populations. In the Sierra Nevada, where mountain sheep, *Ovis canadensis californiana*, have been recently uplisted to endangered status, current management protects remaining sheep by targeting individual mountain lions for removal (S. Torres, personal communication). In the Peninsular ranges, where impacts by mountain lions have been implicated in reductions of the endangered Peninsular bighorn sheep, *O. c. cremnobates* (Boyce et al.[6] 1996), the selective removal of individual mountain lions known or suspected of preying on bighorn sheep is recommended for the recovery of that species (USFWS[11], in press). Thus, when predation by mountain lions threatens the persistence of mountain sheep, removal of these predators may be needed to maintain viable predator-prey systems.

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[10] Nicholson M.C. 1995. Habitat selection by mule deer: Effects of migration and population density. Ph.D. Dissertation, University of Alaska, Fairbanks, Alaska, USA.

[11] USFWS. In press. Draft recovery plan for the bighorn sheep in the Peninsular Ranges (*Ovis canadensis*). United States Fish and Wildlife Service, Region 1, Portland, Oregon, USA.

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FOOD HABITS OF CALIFORNIA CORBINA IN SOUTHERN CALIFORNIA

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We examined the food habits of California corbina, *Menticirrhus undulatus*, by determining dietary changes with size, season, and habitat (protected, semi-protected, and exposed). In addition, we examined how food consumption varied with time of day and tidal stage. Stomach contents were analyzed from 454 California corbina (32–505 mm standard length [SL]) collected between Carlsbad and Hermosa Beach, California from February 1994 to August 1997. Clam siphons were the dominant food of California corbina ≤400 mm SL, whereas clam feet and gills were the primary food of larger fish. Overall composition of the diet varied little among seasons and habitats; clams were the principal prey, followed by crustaceans and polychaetes. California corbina with full stomachs and freshly ingested prey were captured during day and night at high and low tides, indicating feeding occurred throughout the 24-hour cycle. Contrary to our results, past studies found sand crabs, *Emerita analoga*, were the primary prey of larger (>200 mm SL) California corbina. This difference may be a result of opportunistic feeding on fluctuating prey abundances. Although there is some dietary overlap between California corbina and other fishes that occupy sandy, surf-swept habitats, differences in morphology, feeding habits, and microhabitat preferences may alleviate competition for food resources.

INTRODUCTION

The California corbina, *Menticirrhus undulatus*, is an important sport fish in southern California, commonly pursued by shore and pier anglers for its strong fighting qualities and delicate flavor (Roedel 1953, Feder et al. 1974, Oliphant 1992). California corbina range from the Gulf of California north to Point Conception, California and have been reported to reach 3.2 kg (Miller and Lea 1972, Eschmeyer et al. 1983). In southern California, they are found in shallow water (<15 m) habitats along sandy shores of protected and open coasts (Skogsberg 1939, Roedel 1953, Joseph 1962, Allen 1985).

The food habits of California corbina are not fully known. In a brief study by Skogsberg (1939), sand crabs, *Emerita analoga*, were identified as their main prey. More extensive work by Joseph (1962) reported that California corbina <150 mm standard length (SL) fed mainly on clam siphons and small crustaceans, whereas

larger fish fed primarily on sand crabs. However, Allen[1] (1993) found that clams, polychaetes, and small fish were the principal prey of California corbina.

Previous food habits studies of California corbina included few specimens >400 mm SL, reported little information on dietary changes among habitats, and reported no information on seasonal variations in diet. This paper re-evaluates the diet of California corbina from a wide size range and examines dietary changes with size, habitat, and season. In addition, we examined how food consumption varied relative to time of day and tidal stage.

METHODS

Fish Collections

We collected California corbina from 13 locations in southern California from Carlsbad, San Diego County to Hermosa Beach, Los Angeles County between February 1994 and August 1997 (Fig. 1). Collection efforts were sporadic and were concentrated at Belmont Shore in southeastern Los Angeles County. Fish were collected at depths of 1–8 m using 3 gear types: a 30 x 3-m haul seine (1.8-cm square mesh in wings and bag), 4.6 and 7.6-m otter trawls (3.8-cm square mesh body and 1.3-cm square mesh cod end), and a 46 x 2.5-m variable-mesh monofilament gill net (2.5–4.8-cm square mesh). Most fish were collected using the haul seine and otter trawl (66% and 27% of the total specimens, respectively) between 0600 and 1200 hours.

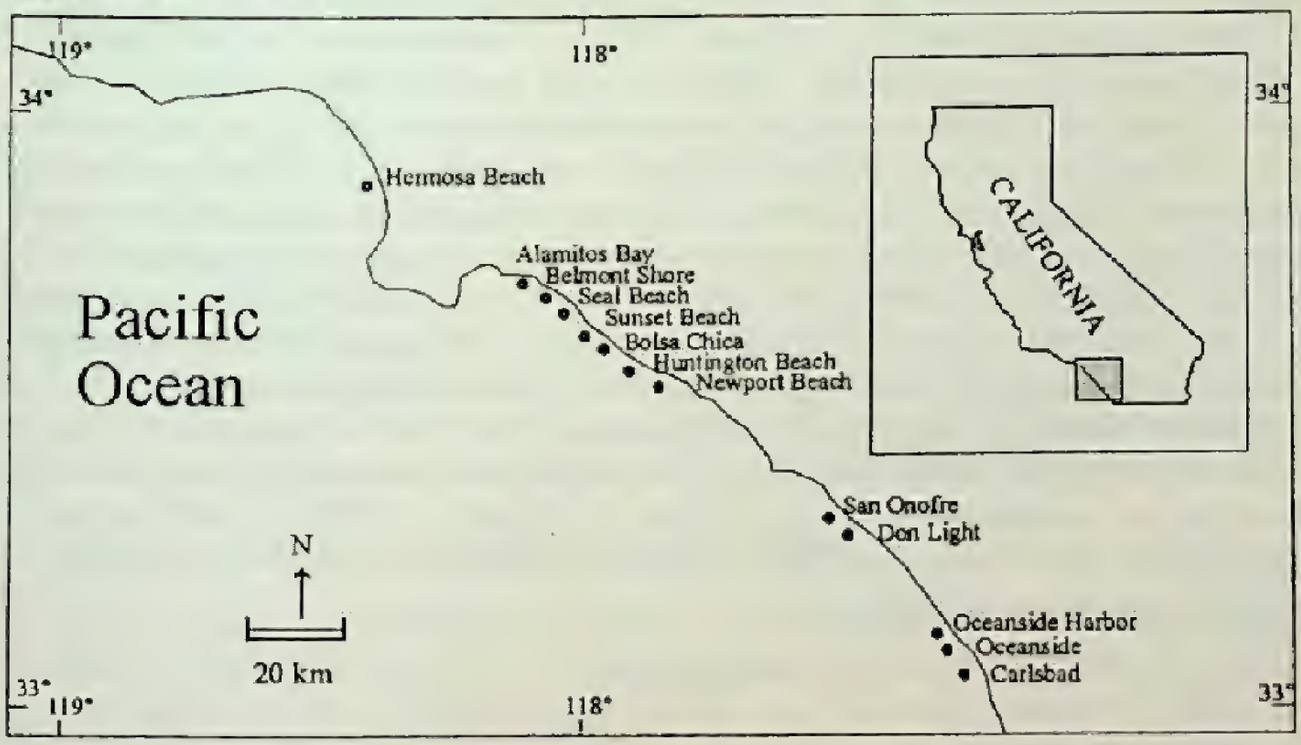

Figure 1. Study area showing locations of collections of California corbina in southern California.

[1] Allen, L.G. 1993. Annual progress report for fiscal year 1992–1993. Bay, Estuarine and Nearshore Ecosystems Studies Contract #FG-2052MR. California Department of Fish and Game, Marine Resources Division, Long Beach, California, USA.

Food consumption relative to time of day and tidal stage was determined by collecting fish with a haul seine over four 3-hour periods (0600–0900 hours, 1200–1500 hours, 1800–2100 hours, and 2300–0200 hours) referred to as sunrise, midday, sunset, and midnight, respectively, in March 1997 at Belmont Shore. Each period was sampled at low water (<0.6 m) and high water (>0.9 m).

Stomach Analysis

Immediately following capture, 2–4 ml of 10% formalin were injected into the abdomen of each fish. Fish were measured to the nearest millimeter SL, weighed to the nearest 0.1 g, and macroscopically sexed. The stomach was removed, fixed in 10% buffered formalin, and transferred to 70% ethanol after 4 days.

Stomachs were opened and assigned a visual estimation of fullness from 0 (empty) to 5 (full and distended). Contents were removed, identified to the lowest practical taxon, counted, blotted on a paper towel, and weighed to the nearest 0.001 g. Wherever possible, only entire organism indicators were counted (e.g., crustacean cephalon or clam foot). Prey items were combined into higher taxonomic categories for analysis. The importance of each prey taxon was determined by computing numerical percentage (N), gravimetric percentage (W), frequency of occurrence percentage (F), and the Index of Relative Importance (IRI = [N+W] F) (Pinkas et al. 1971). Index of Relative Importance ranges from 0 to 20,000.

The IRI was used to compare size, habitat, and seasonal differences in diet. California corbina were grouped into the following length classes: ≤50 mm, 51–100 mm, 101–200 mm, 201–300 mm, 301–400 mm, and >400 mm. The initial 3 length classes were chosen for comparison with Joseph (1962); the last 3 were chosen arbitrarily. Habitat analysis compared the diets of fish according to the habitat where they were collected. Habitat was classified according to overall exposure to wind and swell (Allen 1988) as: protected (Alamitos Bay), semi-protected (Belmont Shore and Oceanside Harbor), and exposed (10 open coast stations from Carlsbad, San Diego County, to Hermosa Beach, Los Angeles County). Seasonal analysis compared the diets of fish collected in winter (December–February), spring (March–May), summer (June–August), and fall (September–November).

Stomach fullness scores were heteroscedastic and not normally distributed. Attempts to rectify this through various transformations were unsuccessful. Therefore, a nonparametric 2-way analysis of variance (ANOVA) with unequal replication on ranked data (Zar 1984) was used to compare the effects of time of day and tidal stage on stomach fullness.

RESULTS

Size Comparisons

We examined the stomachs of 454 California corbina (32–505 mm). Twenty invertebrate families, 3 fish families, and several algal fragments were identified from 424 stomachs containing food. Veneroid pelecypods (primarily bean clams,

Donax spp., and rosy jackknife clams, *Solen rosaceus*) were by far the most important prey (Fig. 2). Veneroid siphons dominated the diet of California corbina <400 mm, whereas veneroid feet and gills were the most important items in larger fish. Anomuran crustaceans (sand crabs; ghost shrimp, *Callianassa* spp.; and hermit crabs, *Pagurus* spp.) were only important in fish >300 mm. Sand crabs were the most important anomuran in fish >400 mm. Gammarid amphipods and mysids were most important in fish ≤50 mm, whereas the importance of cumaceans and unidentified crustacean parts (probably carideans and amphipods) peaked in fish 51–100 mm. Polychaetes were the 2nd most important prey in fish 101–300 mm. Teleosts (gobiids; atherinids; and pipefish, *Syngnathus* spp.) were rarely eaten by California corbina <300 mm and were of minor dietary importance to larger fish. California corbina ≤50 mm had no empty stomachs, but consumed the fewest number of prey types. In contrast, fish >400 mm had the highest percentage of empty stomachs (19%), but consumed nearly twice as many prey types as fish ≤50 mm.

Habitat Comparisons

Veneroids were the most important prey of California corbina in each habitat, followed by anomurans and polychaetes (Fig. 3). In most cases, veneroids were the dominant prey by percent number, percent weight, and percent frequency of occurrence. However, the IRI for veneroids in fish from exposed habitat was much lower than in semi-protected and protected habitat due to a lower frequency of occurrence (62% vs 93% each) and percent weight (32% vs 60% and 49%). Bean clams were the predominant veneroid in fish from exposed habitat, bean and jackknife clams in fish from semi-protected habitat, and the California tagelus clam, *Tagelus californianus*, in fish from protected habitat. Anomurans were of greatest importance to fish in protected habitat, occurring in over 50% of the specimens and accounting for nearly 50% of the weight. Anomurans were the dominant food item by weight of fish in exposed habitat; however, the IRI was relatively low due to a much lower frequency of occurrence. Anomurans in fish from exposed habitat were mostly sand crabs, whereas those from semi-protected and protected habitats were hermit crabs and ghost shrimp, respectively. Polychaetes were the 3rd most important prey in each habitat, and occurred most frequently in fish from semi-protected habitat. The mean number of prey types per stomach varied little among habitats (2.2–2.4).

Seasonal Comparisons

Veneroids were by far the most important prey in each season (Fig. 4). Veneroids had the highest percent frequency of occurrence, percent number, and percent weight in each season except in summer when percent weight of anomurans was higher. This resulted in a lowered IRI for veneroids during the summer. Anomurans were the 2nd most important prey, except during fall, when they were replaced by polychaetes. Sand crabs were the most frequently consumed anomuran in summer, ghost shrimp in winter, and hermit crabs in spring. Polychaetes occurred relatively frequently during all seasons and were of greatest importance during winter. Teleosts

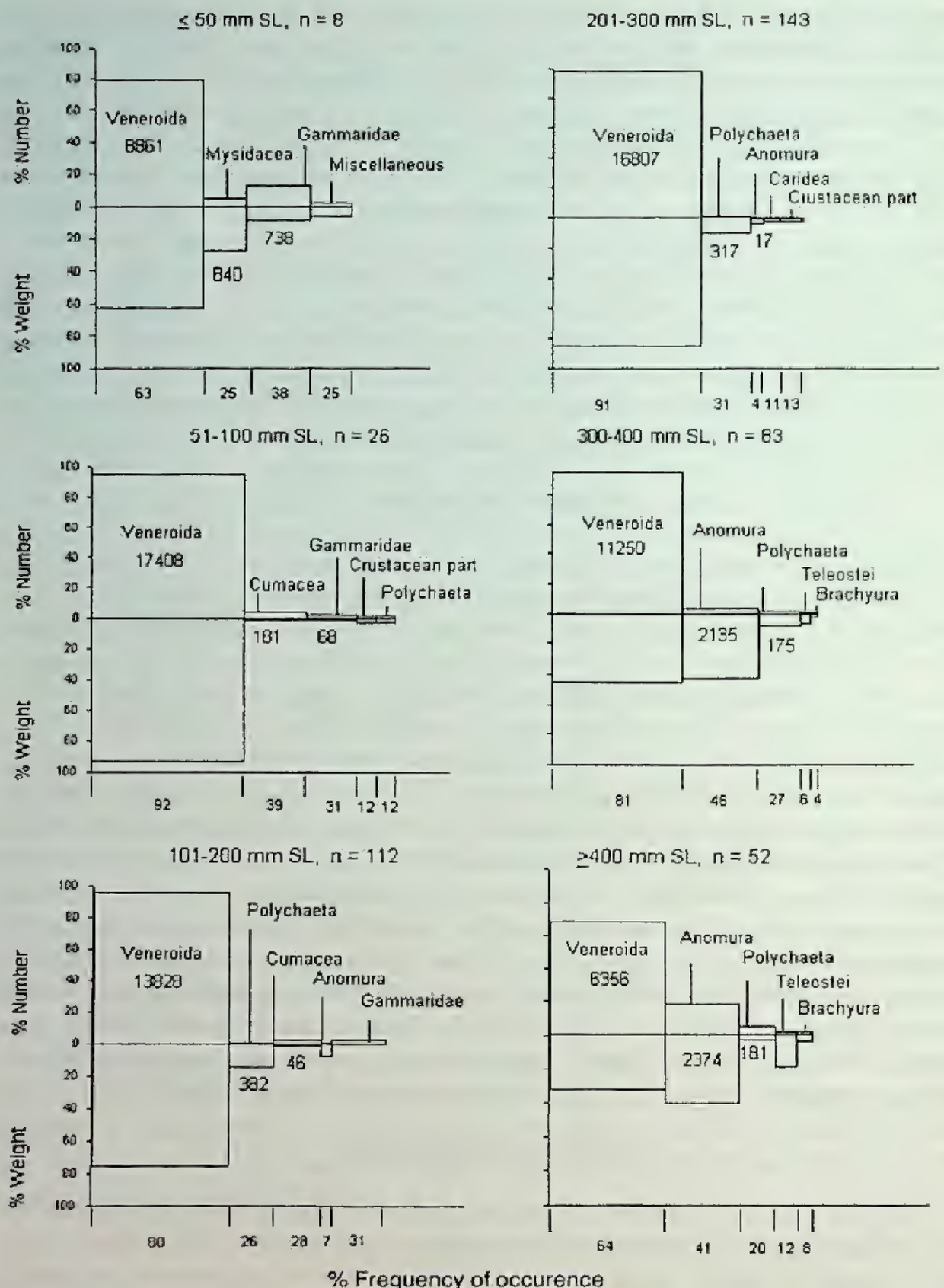

Figure 2. Relative importance of major prey (5 most important in each length class) in the diets of California corbina by length class (modified from Pinkas et al. 1971). The miscellaneous prey category consisted of Tanaidae, Sipunculidae, Nemertea, terrestrial vegetation, and unidentified animal fragments. The Index of Relative Importance for the 3 most important prey categories is listed within or directly beneath bars. Sample size (n) represents the number of stomachs with at least 1 prey item.

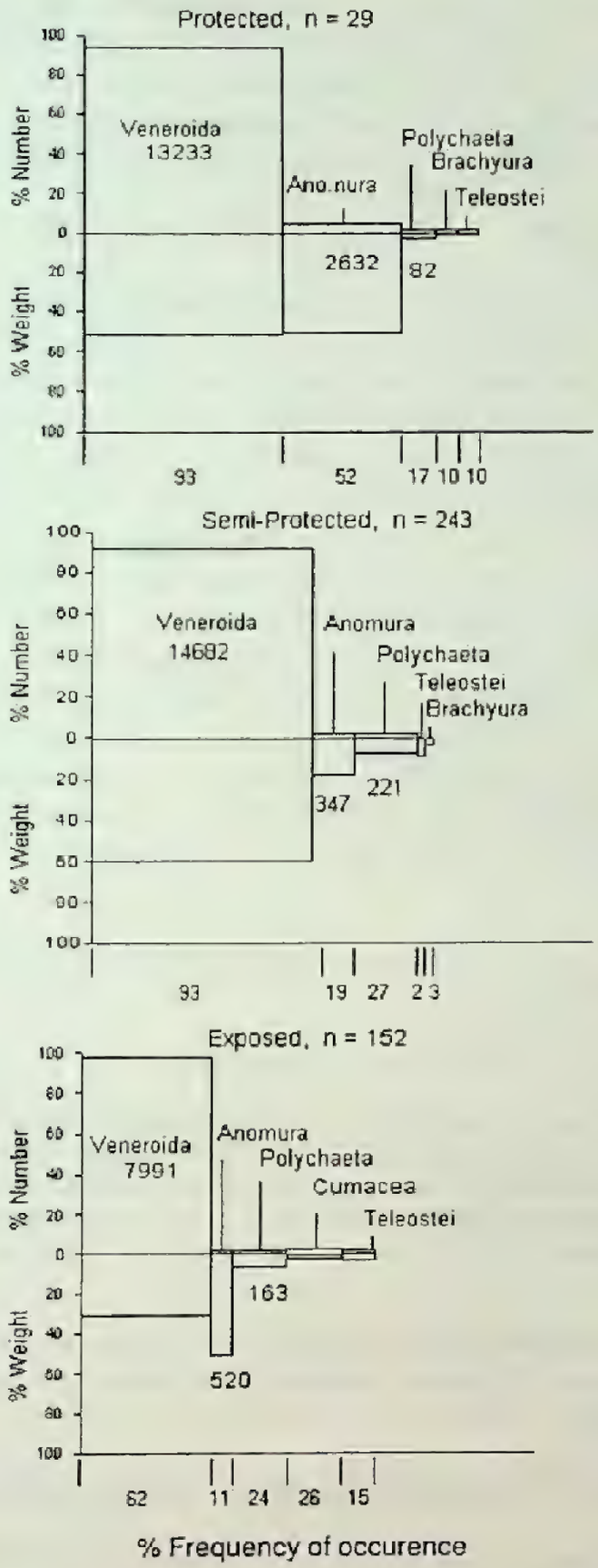

Figure 3. Relative importance of major prey (5 most important in each habitat) in the diets of California corbina by habitat (modified from Pinkas et al. 1971). The Index of Relative Importance for the 3 most important prey categories is listed within or directly beneath bars. Sample size (n) represents the number of stomachs with at least 1 prey item.

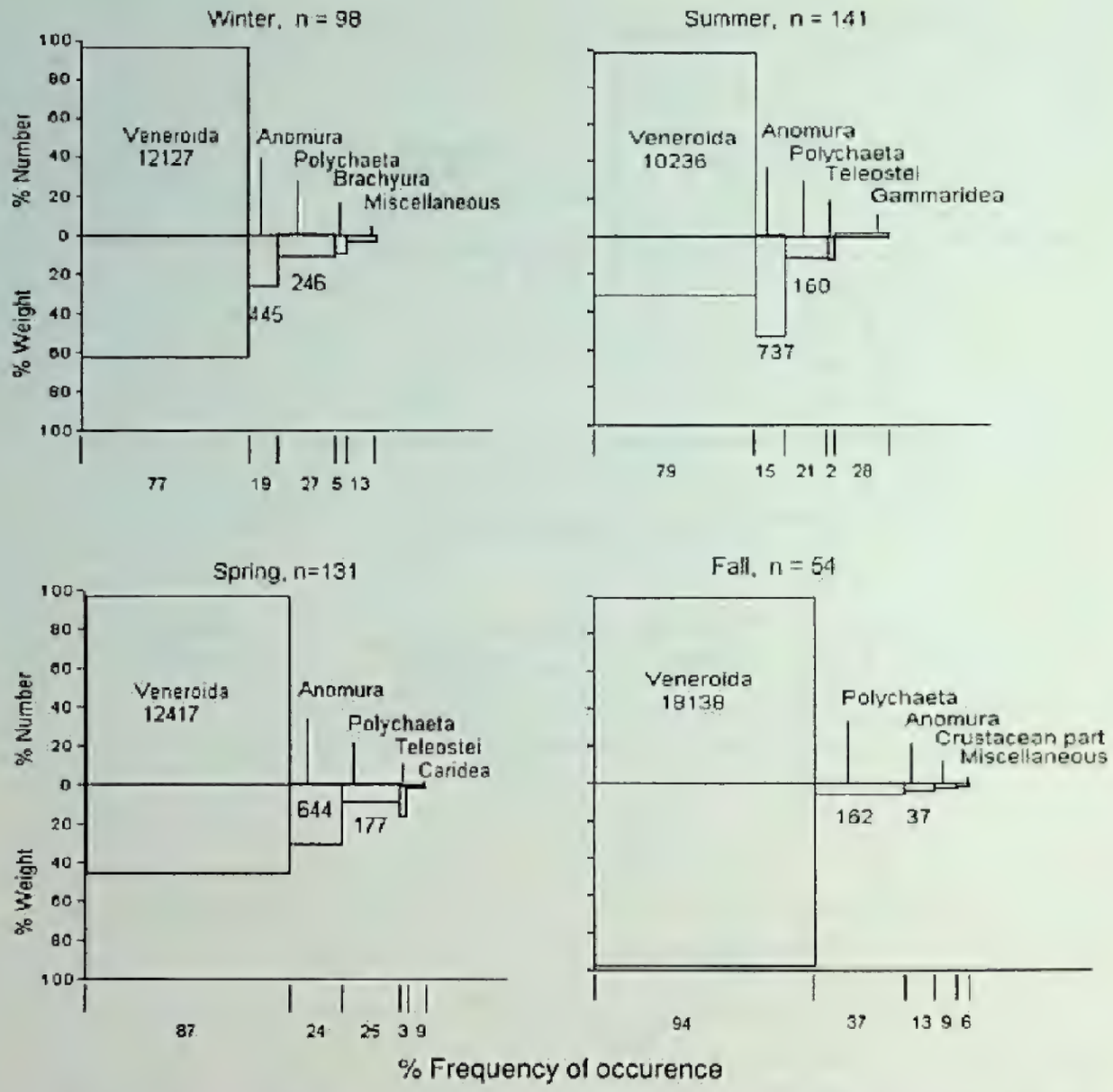

Figure 4. Relative importance of major prey (5 most important in each season) in the diets of California corbina by season (modified from Pinkas et al. 1971). The miscellaneous prey category consisted of Tanaidae, Sipunculidae, Nemertea, terrestrial vegetation, and unidentified animal fragments. The Index of Relative Importance for the 3 most important prey categories is listed within or directly beneath bars. Sample size (n) represents the number of stomachs with at least 1 prey item.

occurred relatively infrequently during all seasons, but were slightly more important during spring and summer. The mean number of prey types per stomach varied little among seasons (2.1–2.5).

Time of Day and Tidal Stage Comparisons

Mean stomach fullness scores varied little; most stomachs were full or nearly full during each combination of time of day and tidal stage (Fig. 5). However, mean fullness scores decreased slightly from midday to sunset and increased slightly from sunset to midnight, regardless of tidal stage. Food consumption peaked at sunrise during low water. However, effects of time of day ($H = 0.03$, $df = 3$, $P > 0.05$) and

tidal stage (H = 1.30, df = 1, $P > 0.05$) on stomach fullness were not significant, and the interaction term also was not significant (H = 1.63, df = 3, $P > 0.05$). Freshly ingested prey were found at each combination of time and tidal stage. Percent of empty stomachs ranged from 0% (midnight during high and low water, sunrise and midday during low water) to 21.4% (sunset during low water).

DISCUSSION

The California corbina is a benthic carnivore, which in southern California feeds predominantly on the soft parts of veneroid pelecypods and whole decapod crustaceans. California corbina have morphological adaptations that aid benthic foraging: a subterminal mouth with small, sharp teeth suited for nipping pelecypod siphons; a pointed snout for digging into the substrate and uncovering infaunal organisms such as sand crabs, ghost shrimp, and polychaetes; a chin barbel which probably aides in locating epi/infaunal prey by acting as a tactile and chemical sensor; and no gas bladder. The chin barbel also probably helps in foraging during low visibility conditions such as those that occur in the surf zone and at night.

We found that as California corbina grow, their diet shifts from veneroid siphons and small crustaceans to larger parts of veneroids and anomuran crustaceans. This shift towards larger prey is not surprising as larger California corbina are probably more effective at uncovering veneroids and infaunal anomurans. However, how California corbina capture the foot of jackknife and tagelus clams is unclear, as these clams usually reside 15–30 cm below the substrate surface (Fitch 1953).

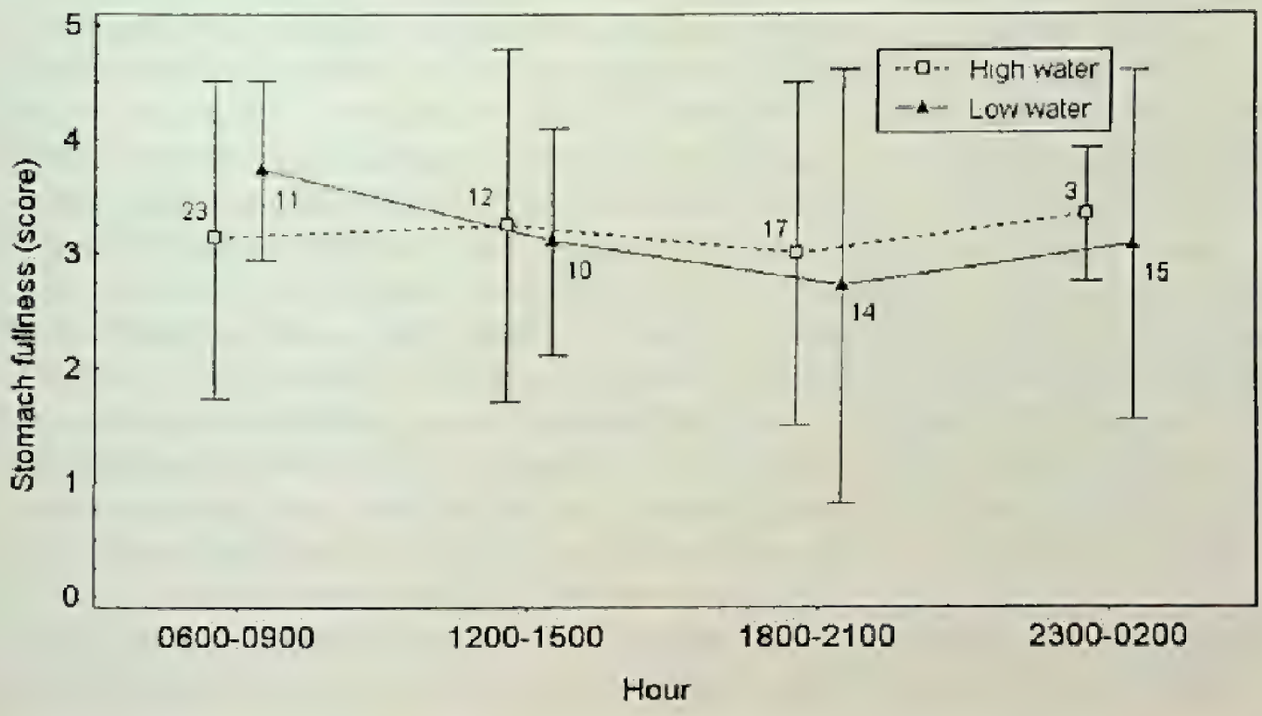

Figure 5. Mean stomach fullness scores (0 = empty to 5 = full and distended) of California corbina during high water (>0.9 m) and low water (<0.6 m) as a function of time of day. Error bars represent ±1 standard deviation. Sample sizes are next to error bars.

Although we collected California corbina from similar locations and depths as Joseph (1962), our findings differed. He found that smaller California corbina (<50 mm) fed mainly on amphipods and mysids, whereas larger fish (>200 mm) fed mainly on sand crabs. We found that California corbina of all sizes fed mainly on veneroids and the frequency of sand crabs in diets of larger fish was much lower than he found (15% vs 74%). These differences are probably a result of temporal changes in the relative abundance of sand crabs and bean clams, as both species experience large temporal population fluctuations in southern California (Coe 1953, Efford 1965, Barnes and Wenner 1968, Cubit 1968). During our study, we observed large numbers of newly settled jackknife and bean clams, whereas sand crabs seemed relatively scarce. Thus, California corbina probably feed opportunistically on veneroids and anomurans and their diet reflects the relative abundance of each prey type.

Regardless of habitat, veneroids were the most important prey for California corbina. However, fish from exposed habitat had a much lower IRI for veneroids. Bean clams were the primary veneroid eaten there and their patchy distribution and small size may explain their lower frequency of occurrence and percent weight. Another difference in diet among habitats was the much higher IRI for anomurans in fish from protected habitat. This was mostly due to the consumption of ghost shrimp, which may be more locally abundant and easier to capture than sand crabs and hermit crabs. Jamie and Kong (1992) noted that the composition of anomurans in the diet of the snakehead kingfish, *Menticirrhus ophicephalus*, a congener from northern Chile, also varied among habitats. One problem with comparing diets among habitats was the possibility of fish feeding in 1 habitat and being captured in another. Another problem was that no California corbina <250 mm were captured from protected habitat. As larger fish generally consumed more anomurans, the increase in anomurans in fish from protected habitat may have been influenced more by fish size than habitat.

Overall composition of the diet varied little throughout the year. The lower IRI value for veneroids in summer may have been due to a lower abundance of adult bean clams, as they may suffer massive post-spawning mortality during summer months (Coe 1955). The increased consumption of teleosts during spring and summer is probably due to the increased availability of juvenile gobiids and atherinids, both of which recruit during those seasons (Feder et al. 1974, Brothers[2] 1975). Although the importance of individual taxa sometimes varied with season, these changes may be due to differences in fish size or habitat. For example, the increase in percent weight of anomurans in California corbina during summer was primarily due to several large fish which had gorged on large sand crabs and ghost shrimp and not because more anomurans were being consumed.

Time of day and tidal stage had little effect on food consumption. Instead, it appeared that California corbina fed throughout the 24-hour period as most stomachs

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[2] Brothers, E.B. 1975. Comparative ecology and behavior of three sympatric California gobies. Ph.D. Dissertation, University of California, San Diego, La Jolla, California, USA.

were full or nearly full and a few contained relatively undigested prey during each 3-hour interval. This finding is supported by the fact that California corbina are commonly caught by anglers during day and night (Skogsberg 1939). However, a larger sample size and information on stomach evacuation rates are needed to more accurately determine how food consumption is related to time of day and tidal stage.

The trophic niche that California corbina occupy in southern California is occupied by several congeners in other temperate and subtropical waters. In the Gulf of Mexico, the Gulf kingfish, *Menticirrhus littoralis*, and the southern kingfish, *M. americanus*, forage in nearshore, surf-swept habitats and feed on benthic fauna (McMichael and Ross 1987, Delancey 1989, Teixeira et al. 1992). McMichael and Ross (1987) also found that juvenile Gulf kingfish and southern kingfish, like California corbina, exhibit an ontogenetic progression in diet; pelecypod siphons, cumaceans, and mysids were the most important prey of small fish (<80 mm SL). In Chile, the snakehead kingfish also forages in nearshore surf-swept habitats and feeds primarily on pelecypod siphons and anomuran crustaceans (Soto et al. 1986, Jamie and Kong 1992).

California corbina diets overlap somewhat with those of other common benthic feeding fishes that occupy nearshore, soft-bottom habitats along the open coast of southern California. The yellowfin croaker, *Umbrina roncador*, forages over sandy, surf-swept habitat (Skogsberg 1939, Roedel 1953, Allen 1985) and preliminary studies have indicated that they feed predominately on teleosts, teleost eggs, brachyurans, carideans, and pelecypods (Allen[1] 1993; J.W. O'Brien and C.F. Valle, California Department of Fish and Game, unpublished data). Unlike California corbina, teleosts and teleost eggs appear to be an important component of their diet and Roedel (1953) reported that live northern anchovy, *Engraulis mordax*, are effective bait for catching yellowfin croaker with hook and line. Also, yellowfin croaker are primarily nocturnal foragers (Hobson and Chess 1986). Yellowfin croaker are morphologically similar to California corbina; however, the presence of a swim bladder enhances mobility (Allen[3] 1982) and may allow yellowfin croaker to feed more efficiently on epibenthic and pelagic prey.

Barred surf perch, *Amphistichus argenteus*, also forage in sandy, surf-swept habitat (Roedel 1953, Carlisle et al. 1960, Allen 1985). Sand crabs are the major component of their diet, although polychaetes, brachyurans, and pelecypods are eaten when sand crabs are scarce (Carlisle et al. 1960). Barred surfperch are morphologically different from California corbina: they have a terminal mouth, a swim bladder, relatively large eyes, and no chin barbel. Barred surfperch are better equipped for feeding on epifaunal prey and foraging in midwater, whereas California corbina are better equipped for digging into the substrate and rooting out infaunal prey.

The round stingray, *Urolophus halleri*, and the bat ray, *Myliobatis californica*, are benthic species commonly found in nearshore, soft-bottom habitats. They feed

[3] Allen, M.J. 1982. Functional structure of soft-bottom fish communities of the southern California shelf. Ph.D. Dissertation, University of California, San Diego, La Jolla, California, USA

primarily on pelecypods, polychaetes, and crustaceans (Ridge[4] 1963, Babel 1967, Talent 1982, Gray et al. 1997). Unlike California corbina, round stingrays and bat rays usually consume the entire pelecypod, and the proportion of pelecypods in the diet increases with fish size. The highest concentrations of round stingrays are found near protected inlets and embayments where water temperatures are elevated and sediments are dominated by soft sand and mud (Babel 1967), whereas California corbina are most commonly associated with sandy, surf-swept areas along the open coast (Skogsberg 1939, Roedel 1953, Joseph 1962, Allen 1985). Bat rays are concentrated at greater depth (3–30 m) than California corbina and, though common in embayments and sandy, surf-swept habitats, bat rays also inhabit rocky reefs and kelp beds (Feder et al. 1974).

Although we did not collect data on prey abundance, comparison of our results with prior studies suggests that California corbina have an adaptable feeding mode which enables them to consume the most available prey. California corbina and many co-occurring species in southern California primarily feed on a small number of abundant prey taxa. Competition for food resources among these fishes may be partially alleviated by their differences in morphology, feeding habits, and microhabitat preferences.

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AGE AND GROWTH OF TUI CHUB IN EAGLE LAKE, CALIFORNIA

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Tui chubs, *Gila bicolor*, were collected from Eagle Lake, Lassen County, California to determine age and growth. The ages obtained from scale and opercular readings matched consistently until age 7. Opercular bones indicated ages up to 35 years, whereas no scale ages were found beyond the 8th year. The pattern of growth was rapid up to the 4th year, after which growth slowed and was very limited after age 8. The age composition and growth pattern of tui chubs in Eagle Lake is probably typical of other alkaline lakes of the Great Basin.

INTRODUCTION

Tui chubs, *Gila bicolor*, are widely distributed in the Great Basin watersheds of California and Nevada and are abundant in many of the terminal lakes of the region. In Eagle Lake, tui chubs are the most abundant fish, providing prey for Eagle Lake rainbow trout, *Oncorhynchus mykiss aquilarum*, and many piscivorus birds. Using scales for age determination, Kimsey (1954) estimated the maximum age of tui chubs to be 7 years. However, Scoppettone (1988) found that large cyprinids and catostomids in Great Basin lakes live much longer than scale ages indicate. He examined an opercular bone from an Eagle Lake tui chub and estimated its age to be 32 years.

The objectives of this study were 1) to determine the age structure of the tui chub population in Eagle Lake, 2) to compare the suitability of opercular bones and scales for aging tui chubs, and 3) to determine if tui chubs live longer than scale ages indicate.

STUDY AREA

Eagle Lake is located about 40 km north of Susanville in Lassen County, California at an elevation of approximately 1,557 m. It is the 2nd largest lake (155 km^2) that lies totally within California. Even though the lake is large, the watershed is relatively small at approximately 1,500 km^2. The lake is composed of 3 interconnecting basins that vary in width from 4 to 7 km (Huntsinger and Maslin 1976). The 2 most northerly basins are shallow, averaging 4–7 m in depth and have very gradual sloping contours. Because of their shallowness, the 2 northern basins are subject to large variations in wetted area relative to small fluctuations in water depth. The southern basin is the largest and deepest (maximum depth 35 m).

Eagle Lake is a terminal lake, although some water leaks into Willow Creek through the Bly Tunnel on the east side of the lake (Moyle et al. 1991). Because Eagle Lake has no natural outflow, the pH is high, varying from 8.7 to >9.5 (8.9 during this study) and increases during periods of drought (University of California, Davis, unpublished data).

Fish species found in Eagle Lake include tui chubs; Eagle Lake rainbow trout; Lahontan redside, *Richardsonius egregius*; Tahoe sucker, *Catostomus tahoensis*; and speckled dace, *Rhinichthys osculus*.

METHODS

Tui chubs were captured using a trap net, gill net, and beach seines from 23 June through 17 July 1998. The trap net had a 1.2 m high x 30.8 m long x 38 mm stretch mesh lead, followed by four 0.9-m diameter circular hoops. The 1st hoop was 1.5 m behind the 2nd frame and the remaining hoops were placed at 0.9-m intervals behind the 1st. The fish were retained in the cod end of a box net, 1.2 m high x 1.8 m wide. The trap net was anchored in place and fish were removed daily in the morning. The trap net was fished at 5 different sites at varying depths and substrates. To target larger tui chubs, a 30.5 m long x 1.8 m wide x 76 mm stretch mesh gill net was set on the bottom at various sites in the southern and middle basins. All tui chubs caught in gill nets were caught in night sets, along with Eagle Lake rainbow trout and Tahoe suckers. To catch small fish, seines of various sizes were used for both day and night sampling, with limited success.

Fish were transported back to the Eagle Lake Biological Field Station where the standard length (SL) to the nearest millimeter and whole body weight to the nearest 0.1 g were measured. A scale sample was removed halfway between the origin of the dorsal fin and the lateral line. Both the right and left opercular bones were removed and boiled for approximately 1 minute to remove the flesh and skin. When possible, sex was determined from examination of gonads.

Each fish was aged by both scale and opercular bone readings. Scales were read at 23x magnification in a microfiche reader. Annuli were identified using the characteristics described by Devries and Frie (1996). The central radius of each opercular bone was examined under a dissecting microscope. Annuli were located by considering an opaque and adjoining translucent band to be 1 annulus (Fig. 1). Two researchers read each scale and operculum and any discrepancies were resolved by joint examination. Where ossification of the centrum of the opercular bone caused difficulty in observing the first 2 annuli, the obscured annuli were exposed by grinding. For verification purposes, the distance between the centrum and first 2 annular rings was measured for fifty 2- to 3-year-old fish to locate the typical position of these annuli. The scales for these fish were also measured to locate the positions of the first 2 annuli.

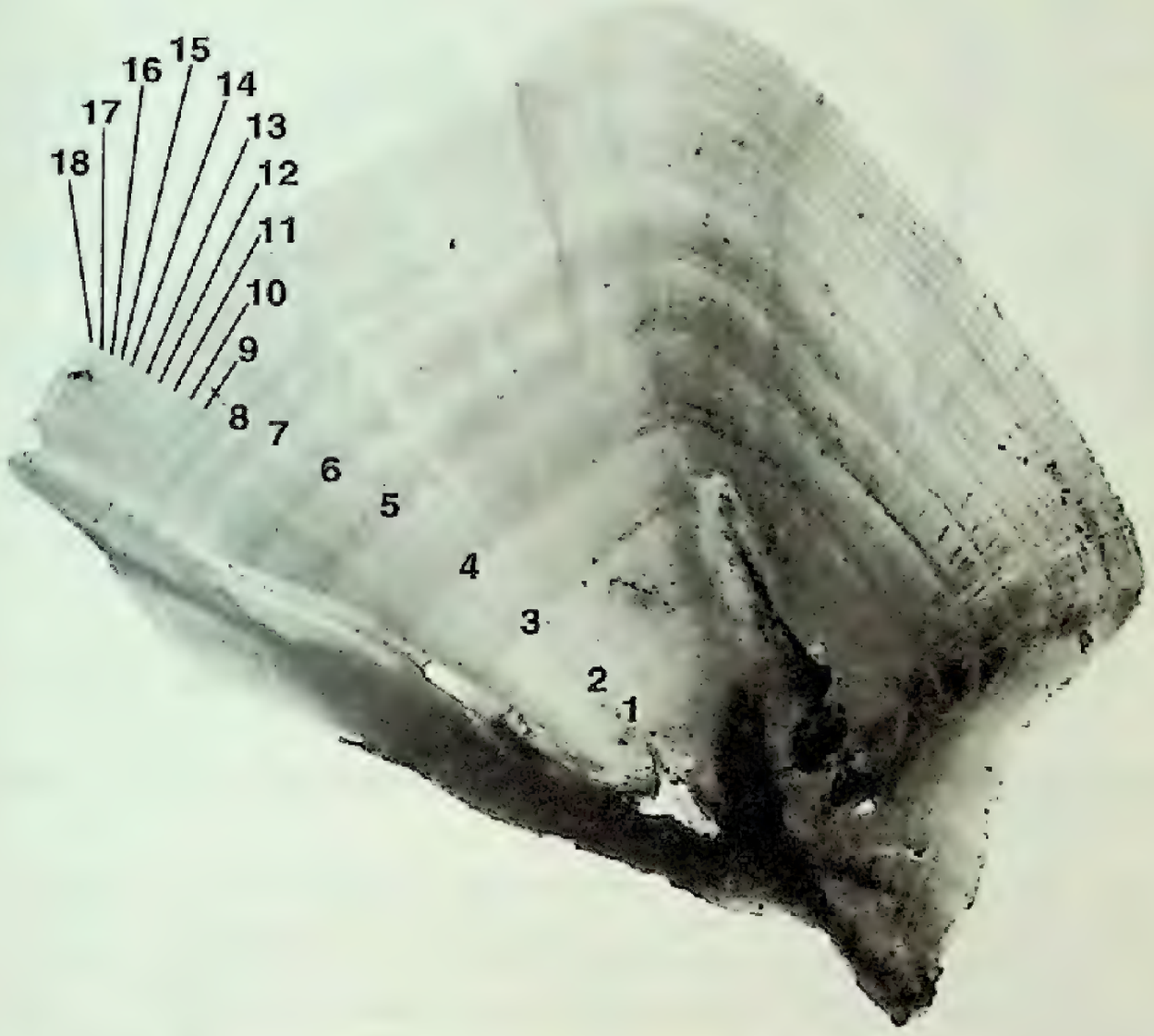

Figure 1. Opercular bone from an Eagle Lake tui chub. Annuli are labelled.

RESULTS

Two hundred and thirty fish, 77–358 mm SL, were examined. Young-of-the-year tui chubs (<77 mm) were not represented because of unusually late spawning in 1998 (P.B. Moyle, University of California, Davis, personal communication). The mean sizes of the fish in each year class to age 7 were: 1, 90 mm (n = 41); 2, 132 mm (n = 48); 3, 163 mm (n = 42); 4, 205 mm (n = 3); 5, 282 mm (n = 10); 6, 290 mm (n = 13); 7, 299 mm (n = 10). A prominent peak in the length-frequency histogram at 330 mm (Fig. 2) coincided with fish aged 8–35 years using opercular ages. The low numbers in the histogram from 200 to 280 mm indicates either 1 or more poor year classes or our inability to catch fish in this size range. The largest fish caught (358 mm) was not the oldest fish in the study. In fish >13 years (n = 9), age did not correlate with length (r = 0.33, n = 14, $P = 0.61$).

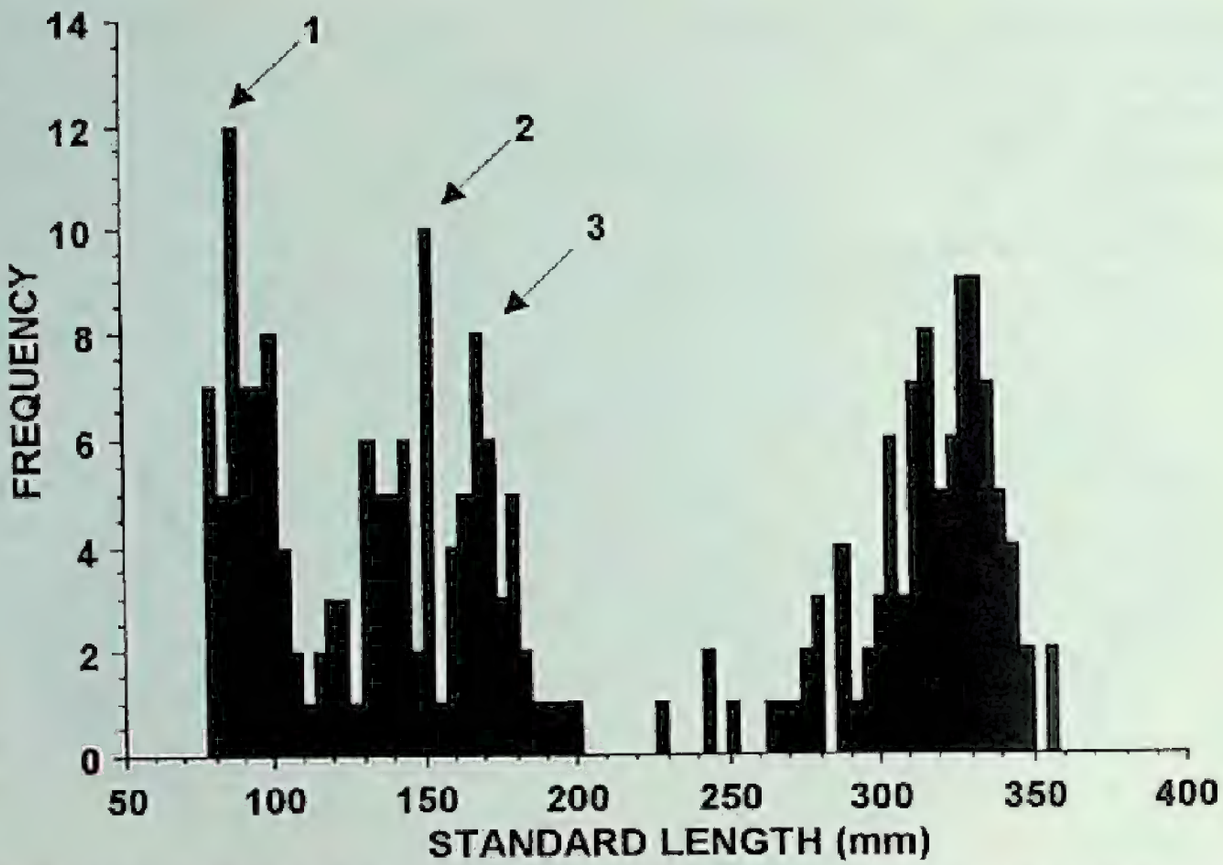

Figure 2. Standard length-freqency distribution of tui chubs from Eagle Lake, 1998. Modes of the first 3 age classes are as indicated.

Opercular ages correlated strongly with the scale ages for fish up to 7 years old ($r = 0.96$, $n = 167$, $P = <0.0001$). When fish over 7 years were included, the correlation between scale and operculum ages was weaker ($r = 0.83$, $n = 230$, $P = <0.0001$). Standard length and the diameter or the operculum were strongly correlated ($r = 0.99$, $n = 230$, $P = <0.0001$).

Plotting standard length against opercular age showed that growth was rapid until 7 years, after which it slowed, and after 10 years was very small (Fig. 3). This growth pattern can also be seen on the opercular bone (Fig. 1).

Comparison of the opercular age-frequency distribution with the scale age-frequency distribution showed that most fish >7 years old were being aged as 7 in the scale readings (Figs. 4 and 5). Both age-frequency distributions had high frequencies of ages 1 to 3, low frequencies of age 4, and increasing frequencies of ages 5–7. But in the scale age-frequency distribution the frequency of age-7 fish was much higher than in the opercular distribution and there were few age-8 fish and no fish older than 8 years. In contrast, the frequency of fish >8 years was high in the opercular distribution.

DISCUSSION

Tui chubs in Eagle Lake appear to live much longer than previously thought by Kimsey (1954) who found the maximum age to be 7 years from scale examination. While ages in this study and Kimsey's were not directly validated, a number of lines

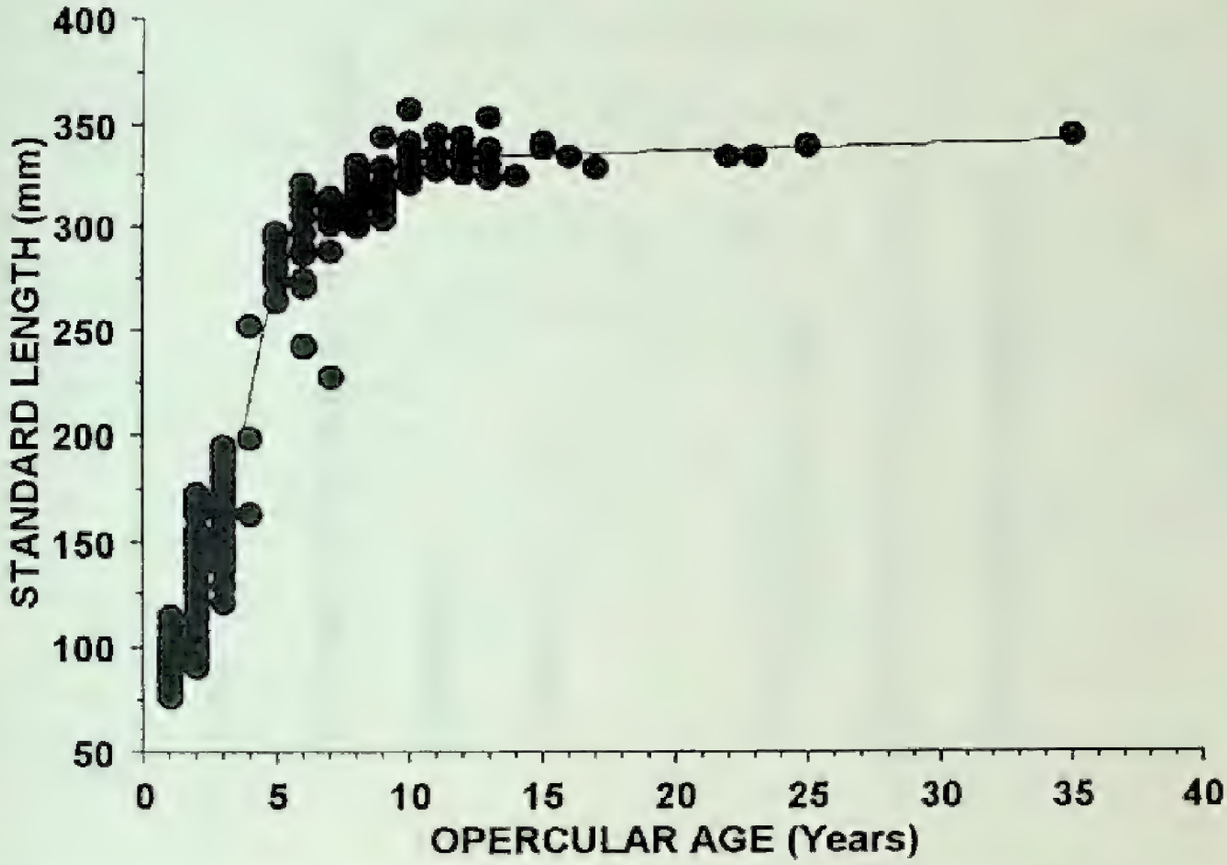

Figure 3. Standard length-opercular age relationship of tui chubs from Eagle Lake, 1998.

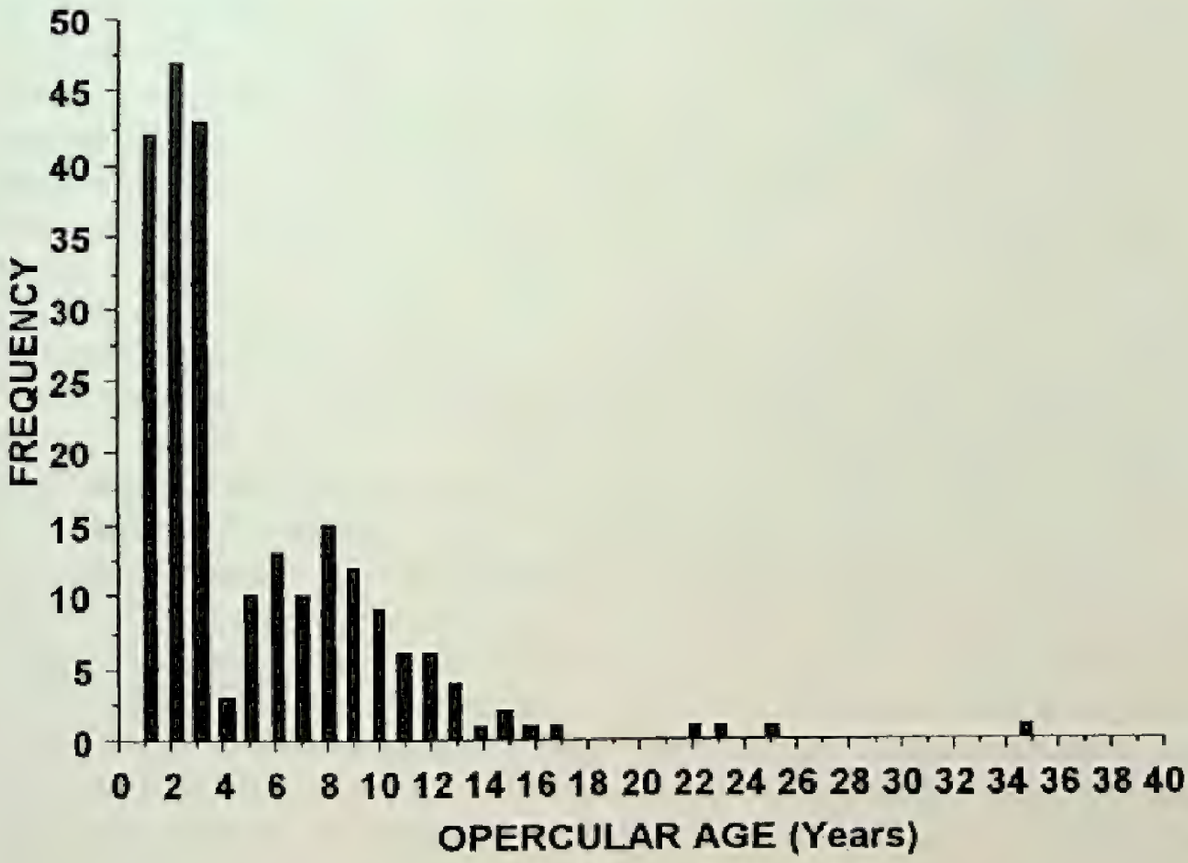

Figure 4. Opercular age-frequency distribution of tui chubs from Eagle Lake, 1998.

Figure 5. Scale age-frequency distribution of tui chubs from Eagle Lake, 1998.

of evidence indicate that the opercular ages are correct. First, the growth pattern of the opercular bone corresponds to the standard length of the fish. Second, the length-frequency histogram showed clear peaks for age classes 1–3, which matched the peaks on the opercular age-frequency histogram. Third, scales and opercular bones produced highly correlated ages for the first 6 years. This strongly suggests that the rings are annular. Fourth, Scoppettone (1988) found that opercular bones were reliable indicators of age and growth of fish in Pyramid Lake, Nevada, which has similar environmental conditions to Eagle Lake. He was able to follow 2 cohorts of cui-ui, *Chasmistes cujus*, and found the opercular bone to be accurate for aging fish older than 30 years. Using opercular bones, Scoppettone (1988) found that many large cyprinids and catostomids from western North America were long lived.

The tui chub growth pattern found in Eagle Lake is similar to that found by Scoppettone (1988) in the cui-ui, with 3 periods of growth over the life span of the fish. The first 2 growth periods are also similar to what Kimsey (1954) found when he compared standard length to scale age. The first 3 or 4 years showed wide bands of opercular bone growth which then became narrower, indicating somewhat slower growth until the 7th or 8th year (Fig. 1). After this, the bands become very narrow, indicating very slow growth. Kimsey (1954) indicated that tui chubs in Eagle Lake become sexually mature at age 3. From ages 3–7 growth slows, suggesting that although the chubs are investing energy in gonadal growth, they are still budgeting for somatic growth so that they will reach the size needed for maximum fecundity. After 7 years there is little somatic growth, as they invest most of their resources in reproduction (Moyle and Cech 1996).

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THE FIRST EASTERN PACIFIC REPORT OF THE SHARPTAIL MOLA, *MOLA LANCEOLATA* (TETRAODONTIFORMES: MOLIDAE)

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On 25 May 1999, a specimen of sharptail mola, *Mola lanceolata* (Liénard, 1840), was found stranded on a beach near El Comitán, at the head of Ensenada de La Paz (24°7'N, 110°25'W), Bahía de La Paz, Baja California Sur, México. This is the first valid record of the sharptail mola in the eastern Pacific Ocean. The previous known range of this rarely seen fish was nearly worldwide in warm waters, including both sides of the Atlantic Ocean, the Indo-west Pacific, and the Red Sea (e.g., Liénard 1840, Fraser-Brunner 1951, Bigelow and Schroeder 1953). Occasionally this species has been cited as *Masturus oxyuropterus* (Bleeker, 1873) (Eschmeyer et al. 1998).

Our specimen measured 1,950 mm total length (Fig. 1). The following measurements (in mm) were made in situ: preclaval length (defined by Martin and Dewry [1978], as a substitute for standard length) 1,630; head length 490; horizontal orbital diameter 75; preorbital length 160; preanal length 1,150; greatest body depth (dorsal fin origin to anal fin origin) 970; dorsal fin height 610; anal fin height 570; distance from dorsal fin tip to anal fin tip 2,070; pectoral fin length 195; horizontal length of clavus (posterior fin of molids lacking hypural support and including rays apparently derived from dorsal and anal fins and, in *Masturus*, with a central lobe with probably true caudal rays) 320. Fin ray counts were: dorsal 19, anal 16, pectoral 9, and clavus 19. The vertebral count was 16 (8+8).

The fish was beginning to decompose and as we had no container large enough for it, we photographed it, measured it, and wrapped it in a fishnet to decompose in seawater for a week. The remaining spongy bones were deposited in the fish collection of the Centro de Investigaciones Biológicas del Noroeste (CIBNOR 0501).

Although this is the first published report on the sharptail mola from the eastern Pacific Ocean, 7 collections of larvae and juveniles listed as *Masturus lanceolata*, mostly from Hawaiian waters, are in the Scripps Institution of Oceanography Fish Collection (Table 1). The earliest dates from September 1948.

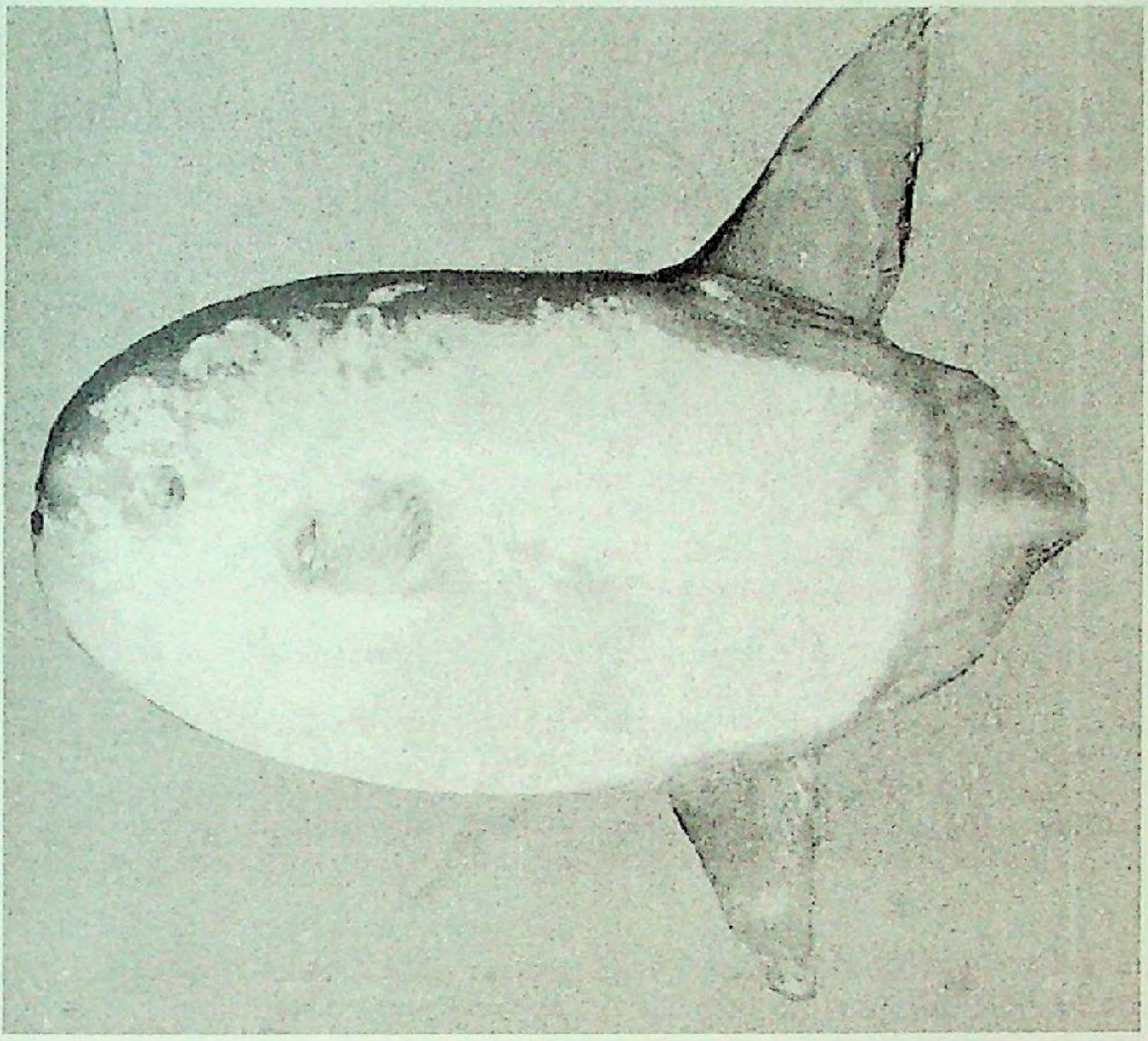

Figure 1. Sharptail mola, 1,950 mm TL, stranded at Ensenada de La Paz, Gulf of California, México, 25 May 1999.

Table 1. List of larvae and juveniles of sharptail mola in the Scripps Institution of Oceanography Fish Collection.

Catalog No.	Date	Latitude, Longitude	No. Specimens	Size Range (mm)
SIO 58–128	Sept. 1948	24°00'N, 167°00'W	19	5.5–46.0
SIO 77–218	12 June 1976	6°30'N, 139°00'W	1	30.0
SIO 77–251	15 Jan. 1976	6°33'N, 127°1'W	1	5.0
SIO 82–61	8 Aug. 1982	28°00'N, 154°00'W	1	15.5
SIO 89–79	14 Aug. 1986	5°00'N, 110°00'W	2	124.0–139.0
SIO 95–97	18 Jan. 1968	3°58'N, 104°17'W	3	4.0–5.0
SIO 95–109	26 Jan. 1968	5°39'N, 97°45'W	1	3.0

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